diagrams of those systems, also of the peculiar properties of the three-phase line, has engaged my attention for a considerable time. I hope to refer to this later.

TABLE II.

Triple point pressures of the components.

Component.	Temp.	Duration of the experiment	Mols in 100.000 L		Pressure in mm. of Hg.
			found	mean	
$pC_6H_4Cl_2$	53°.0	3 hours	41.7	41.9	8.53
	53°.0	6 „	42 1		
$pC_6H_4Br_2$	87° 2	4 hours	39.5	40.5	9.10
	87.°2	4 „	41.5		

TABLE III.

Tension on the three-phase line and composition of the gas.

No.	Experiment temp.	Duration of the experiment in hours	mol. % of $pC_6H_4Br_2$ in the mixture used	mols in 100.000 L		Pressure in mm. of mercury	Composition of gas-phase in mol. % $pC_6H_4Br_2$	
				found	mean		found	mean
1	58.5°	10½	26.1	46.9	46.6	9.64	7.8	8 5
2	58.5°	12½		46.3			9.2	
3	62.0°	7½	42 0	50.5	50 3	10 52	12.6	12 2
4	62.0°	7	41.9	50.1			11 8	
5	69.8°	10	59.5	55.5	55.9	11.96	23 6	23 7
6	69.8°	12		56 4			23.8	
7	76 0°	10	75.2	57 1	57.0	12.39	26 7	26.8
8	76.0°	25		56.9			26.8	
9	83.0°	21	93.0	53.6	54 0	12.00	45.8	46.0
10	83.0°	23		54 3			46.2	

Utrecht, VAN 'T HOFF-laboratory. May 1910.

Chemistry. – "*On the alkaloid content in the leaves of the Cinchonas.*" By P. VAN LEERSUM.

(Communicated in the meeting of May 28, 1910).

Historical review.

In his report as to the alkaloid content of the bark and the leaves of the cinchona trees cultivated in Java (Jan. 1, 1864), JUNGHUHN states that according to the Calcutta Gazette Supplement (Aug. 15, 1863) Dr. TH. ANDERSON had prescribed successfully decoctions of the fallen leaves of *C. Succirubra* for fever in the hospital at Darjeeling.

On treating an acid decoction of these leaves with sodium carbonate he obtained small crystals which he thought might be quinine sulphate

JUNGHUHN proceeds: "Although Dr. J. E. DE VRIJ has already analysed cinchona leaves from Tji-Bodas and stated in his report that he has not found a trace of alkaloids therein still I have thought it necessary to test once more carefully the leaves of all our local cinchona species as to their possible alkaloid content, and this was carried out in exactly the same manner as the assay of the barks."

JUNGHUHN's process was as follows:

The leaves with the stalks adhering were cut up into small pieces and dried at 100° until no further loss of weight took place. 40 grams of the dry sample were now boiled gently for an hour with 10 times the weight of acid water (1 part of sulphuric acid to 300 parts of water), the water lost on evaporation being constantly replenished.

The decoction was filtered through flanel and the mass boiled once more with acid water and then twice with plain water. The united filtrates contained in a cylindrical glass were neutralised with ammonia and treated with a solution of tannic acid and the precipitate was collected on a filter.

JUNGHUHN was of opinion that the precipitate consisted of a bitannate of quinine and chinchonine and treated it with calcium hydroxide and alcohol. The alcoholic filtrate was evaporated in a little basin and the residue dissolved in water containing sulphuric acid and filtered. The acid liquid occupying not more than 7 or 10 cc. was collected in a beaker and rendered alkaline with ammonium carbonate which caused the alkaloids to be precipitated as white flakes. The precipitate was collected on a weighed filter, dried and weighed.

The alkaloid content found by JUNGHUHN in this manner amounted in the leaves of *C. Pahudiana* to 0.420 %, of *C. lancifolia* to 0,220 %, of *C. Calisaya* to 0.587 and of *C. succirubra* (fallen, partly green, partly reddish-brown and withered) to 0.520 %.

After JUNGHUHN, it was DE VRIJ who was engaged in the investigation of the leaves of the cinchona tree.

DE VRIJ first attempted to extract the powdered leaves with dilute hydrochloric acid but the results obtained were very unfavourable.

He then operated as follows:[1])

The powdered leaves were mixed in a spaceous porcelain dish with one-fourth part by weight of calcium hydroxide and then made with water into a thin paste which was left for some days with

[1]) Kinologicol studies: *Ned. Tijdschr. Pharm. Chem.* and *Toxic. 1896.*

occasional stirring until the whole mixture had assumed a dark-red colour.

The object of this tedious repeated stirring was to convert the large quantity of kinotannic acid present in the leaves, which was probably the cause of the failure of the hydrochloric acid extraction, into cinchona red by the action of the air and the excess of calcium hydroxide and this object was fully obtained. The mixture was now dried and extracted with alcohol which was afterwards recovered by distillation. The residue was then warmed with dilute acetic acid and the calcium precipitated with ammonium oxalate. The liquid was filtered, a large quantity of chlorophyl being left on the filter. The perfectly clear filtrate had a very pale yellow colour and yielded with ammonia an abundant, voluminous, but very light precipitate, the weight of which, after washing and drying amounted to only 0.162 %. It was a deep yellow powder which did not melt on the waterbath, but dissolved in alcohol to a brown solution. This was again evaporated and the residue converted into acid sulphate; a comparatively large quantity of a reddish-brown substance remained insoluble and was removed by filtration. To the almost colourless filtrate was now added a solution of iodine in potassium iodide, which yielded a fairly abundant precipitate. This was collected on a filter. washed, dried and dissolved in a little warm alcohol.

In this liquid Prof. BEHRENS could not observe microchemically, a trace of any kind of crystalline herapathite [1]) from which it follows that the said precipitate is a compound of amorphous alkoloid with JHJ and H_2SO_4.

The conclusion arrived at by DE VRIJ from this investigation of the cinchona leaves is that they contain one (or more) amorphous alkaloids which are afterwards converted in the living plant into crystalline alkaloids such as occur in admixture with more or less amorphous alkaloid in the cinchona barks.

According to MOENS[2]) the leaves contain very little or no alkaloid and J. C. HOWARD[3]) found once a little in *Succirubra* leaves, but afterwards none in 20 pounds of the same.

From fresh *Succirubra* leaves BROUGHTON [4]) also obtained only 0.0041 % of alkaloid of which 0.0016 % was quinine; from the dry leaves 0.019 % of alkaloid of which 0.008 % was quinine; from *Ledgeriana* leaves MOENS obtained only traces.

[1]) Kinological Studies. Ned. Tijdsch. Pharm. Chem. and Toxicol. 1899 p. 104

[2]) The Cinchona culture in Asia 1854—1882.

[3]) Ph J. 1878 p. 541.

[4]) Blue book 1870 p. 278.

In fresh *C. officinalis* leaves, BROUGHTON found 0.0035 % of alkaloid of which 0.0015 % was quinine.

Owing to the very divergent results obtained in the analysis of the cinchona leaves, LOTSY[1]) decided to investigate the leaves of the cinchona tree once more and to ascertain whether these organs play also a role in the formation of the alkaloid.

The *modus operandi*: employed by LOTSY is described as follows:

The parts of the leaf (to the left and the right of the midrib) were cut up into very small squares and boiled for half an hour in alcohol containing $^1/_2$ % of HCl (20cc. of strong hydrochloric acid per litre). This took place on the waterbath in small ERLENMEYER flasks closed with a cork fitted with a long tube serving as a reflux condenser. The alcohol was then poured into small porcelain dishes placed on the waterbath and evaporated nearly to dryness. Water was poured into the dishes and the solution again evaporated nearly to dryness in order to be certain that all the alcohol had been expelled.

More water was again added, the solution was filtered and the filtrate collected in a separatory funnel. After being rendered alkaline with KHO, the liquid was shaken with chloroform, which was then evaporated in a watchglass on the waterbath.

The residue was then taken up with water containing $^1/_2$ % of HCl and thoroughly rubbed with a glass rod to detach the resinous matters from the watch-glass. The solution was passed through a miniature filter and the filtrate then used for the alkaloid reactions. LOTSY (l.c.) now arrives at the following conclusions.

1. The amount of alkaloid present in the leaves of a *Cinchona succirubra* and in those of a *Chinchona Ledgeriana* is many times more than sufficient, when transported to the bark regularly, to form the amount of alkaloid present therein pg. 8.

2. *Cinchona succirubra* leaves can part with the whole of their alkaloid supply in 12 hours p. 99.

3. The extent of the formation and migration of the alkoloid is influenced by the weather.

4. The alkaloid disappearing from the *Succirubra* leaf is transported to the stem pg. 18.

5. The alkaloid which is found afterwards in the same leaf has been generated by the leaf itself.

And on pg. 19 it is further stated.

"We may, therefore, come to the final conclusion, without being unduly speculative, that in the cinchona trees the alkaloid is formed

[1]) J. P. LOTSY. Physiological experiments carried out with *Cinchona Succirubra*. Communication from the Government Botanical Gardens 36 1899.

in the leaves and from thence migrates to the stem, where it is retained either in its original form or in that of a new compound (thus forming an alkaloid different from that derived from the leaves)" and further:

"It stands to reason that these experiments do not yet exclude the possibility of a formation of alkaloids in the bark itself but — looking at the experiments and the arguments held — we may safely assume that it is insignificant, in comparison to what is formed in the leaves and from thence transported to the stem.

Experimental.

In my own investigation the above mentioned process of LOTSY was tried first but this did not prove satisfactory, for if, according to LOTSY's directions, potassium hydroxide is added to an acid solution of cinchona alkaloid still containing impurities the first drops throw down no alkaloid, but all kinds of impurities and, considering the large number of substances occurring in the leaves, which pass together with the alkaloid into the different solvents, this separation of impurities is not trifling. It would have been better (and the results obtained would have looked quite different) not to have used an acid solution for testing of alkaloids, but to have neutralised the liquid (or rendered the same faintly alkaline) in order to get rid of the impurities.

Then, there would have been no risk of failing to obtain a precipitate by adding an insufficiency of alkali to a too strongly acid solution, and all danger of a coprecipitation of foreign matters would have been avoided.

The following process was therefore employed.

24 grams (or less) of the leaf powder (sieve B 40) were mixed with 12 grams of calcium hydroxide and then made into a coarse mass with 8 grams of 15% sodium hydroxide and 12 grams of ammonia. This mass was shaken for 3—4 hours with 600 cc. of ether and from the clear, green solution 500 cc. (= 20 grams of leaf) were taken. Before proceeding to distillation 10 cc. of 1% sulphuric acid and 20 cc. of water were added and the mixture was thoroughly shaken.

The ether was now distilled off very slowly.

If the ether is evaporated before addition of the acid water, the large quantity of vegetable fat prevents a thorough contact between the acid and the alkaloid; and a loss occurs.

When manipulating like this in the analysis of leaf rib and leaf

stalk all the impurities were separated in a pulverous form, and the washing caused no trouble whatever.

When testing mesophyll particularly that of the *Ledgeriana* leaf, in which occurs more vegetable fat, this did not go so readily, and traces of alkaloids were retained.

The acid yellow coloured liquid was thoroughly shaken in the flask with a few pyropes and filtered.

The filtrate was collected in the separatory funnel and the flask containing the insoluble impurities washed repeatedly with water, until the washings were no longer acid.

After being rendered alkaline, the liquid was shaken four times in succession with 50 cc. of ether, and each time, the flask, in which the extracted liquid was collected before being returned to the funnel, was rinsed with 50 cc. of ether.

The ethereal liquids were collected in another separatory funnel and left at rest for some time to allow any alkali and suspended impurities to deposit.

The deposit formed was then removed with water and the washing continued until the water was no longer alkaline.

The pale yellow coloured ether, containing the alkaloid in solution, was first shaken with 10 cc. of $N/_{10}$ hydrochloric acid and a little water.

After the two layers had separated, the acid aqueous solution containing the alkaloid was collected in a beaker and the ether was again shaken four times in succession with pure water.

After the ether dissolved in the acid water had evaporated spontaneously the excess of acid in the liquid (measuring about 250 cc.) was titrated with $N/_{10}$ alkali, using haematoxylin as indicator.

The observation of the change from yellow into green requires some practice, but still the end reaction is plainly perceptible.

The above method, though tedious, gave good results and the following analyses of bark show that the alkaloid is completely extracted. Assay *A* is made by the method described and *B* by a totally different method of bark analysis.

Sample 1.

A. 7.70 % of quinine sulphate.

B. 7.60 % „ „ „

Sample 2.

A. 5.00 % of quinine sulphate.

B. 4.98 % „ „ „

Sample 3.

A. 5.40 % of quinine sulphate.
B. 5.40 % „ „ „

Sample 4.

A. 6.85 % of quinine sulphate.
B. 6.84 % „ „ „

The subjoined figures were obtained, by the method described, in the assay of *C. succirubra* leaves without mid-rib.

1st analysis 0.739 % of total alkaloid.
2nd „ 0.721 % „ „ „
3rd „ 0.739 % „ „ „
4th „ 0.750 % „ „ „

Now, in order to ascertain whether the results obtained by LOTSY [1] were correct, the process described by him was followed, that is to say, that two halves of the same leaf were always used for the research. These halves were always longitudinal ones.

They were obtained by cutting exactly along the mid-rib of the leaf. In this manner the leaf was divided into two unequal parts one with and one without mid-rib.

The piece without mid-rib was tested at once, that with the mid-rib remained attached to the tree. At the end of the experiment the mid-rib was removed and the remaining half of the leaf was then tested.

The pieces of leaf to the left and the right of the same mid-rib were in this manner compared with each other and LOTSY obtained the following results (l.c. pg. 9).

6 p.m. 18 Sept. '99		6 a.m. 19 Sept. '99.
N°. 284	full	empty
„ 285	„	„
„ 286	„	„
„ 287	„	„
„ 288	„	„
„ 289	„	„
„ 291	„	„
„ 292	„	„
6 a.m. 21 Sept. '99		6 p.m. 21 Sept. '99,
N°. 305	full	empty
„ 308	„	„
„ 310	„	„

[1] l.c. p. 4.

In order to ascertain the possible influence of light and darkness, I placed on 19/8 '08 a *Ledgeriana* tree about five years old entirely under a box lined with lead foil but first of all, from a large portion of the well developed tree one half of each leaf was removed, leaving the mid-rib attached to the other half.

After removing the box on 3/9 '08 the second half of the leaves was examined.

Result:

I. *A.* Investigation of the part without mid-rib viz. that removed from the leaf before covering with the box.

1st half. Total alkaloid 0.410%.

B. Investigation of the other half of the leaf viz. that which had been excluded from the light for 16 days with its rib.

2nd half. Total alkaloid 0.430%.

II. *A.* Investigation of the part without mid-rib, viz. the part removed from the leaf before covering with the box.

1st half. 0.412% of total alkaloid.

B. Investigation of the other half of the leaf, viz. the part excluded from the light for 16 days with the rib.

2nd half. 0.410% of total alkaloid.

Leaf rib and leaf stalk 0.695% of total alkaloid.

In addition to the above experiments the following comparative experiments were made.

A cultivating bed planted with Ledgeriana seedlings was divided into two plots *A* and *B*.

The plants in plot *A* were on 19/8 '03 excluded from the light by means of a box lined with lead foil, but beforehand one-half of the leaf was removed and investigated.

Plot *B* remained uncovered and, therefore, kept growing under normal conditions but one-half of the leaf was also removed and tested.

On 4/9 '08, or 16 days afterwards, the box was removed and the other half of the leaf was tested, also the 2nd half of the leaf from plot *B*.

Plot *A* (leaves, the first half tested 19/8, the 2nd half after having been in darkness for 16 days).

Plot *A*. 1st half of the leaf. Total alkaloid 0.508%.
2nd „ „ „ „ (darkness) Total alkaloid 0.530%.

Plat *B*. 1st half of the leaf. Total alkaloid 0.447%.
2nd „ „ „ „ (light) Total alkaloid 0.460%.

If now, LOTSY's theory were correct that the alkaloid in the Cinchonas is a product of assimilation, therefore a substance formed like amylum

in the leaf and ready to be conveyed to the stem, the leaves which have been excluded from the light for a considerable time ought no longer to contain alkaloids or, in any case, much less than the leaf under normal conditions.

The above results, however, show the reverse. Moreover, according to the said theory, none or little alkaloid ought to be present in the fallen leaves and this should have been transported previously to the fall either entirely or for the greater part.

The subjoined analyses, however show the reverse.

Mesophyll of plucked, still living, green Succirubra-leaves. I.	Mesophyll of fallen Succirubra leaves of the same tree. II.	Leaf stalk and mesophyll of the still living green leaf. III.	Leaf stalk and mesophyll of the fallen Succirubra leaf. IV.
Total alkaloid in proc.	Total alkaloid in proc.	Total alkaloid in proc.	Total alkaloid in proc.
a. 0,728	*a.* 0,739	*a.* 1,01	*a.* 1,23
b. 0,739	*b.* 0,721	*b.* 0,997	*b.* 1,15
	c. 0,739		
	d. 0,750		
Id. of Ledgeriana.	Id. of Ledgeriana. (same trees)	Id. of Ledgeriana.	Id. of Ledgeriana.
a 0,410	*a.* 0,420	*a.* 0,500	*a* 0,647
b. 0,440	*b.* 0,440	*b.* 0,580	*b.* 0,615
c. 0,390			
d. 0,408			

As, however, the possibility is not excluded that the tree when covered for 14 days or a month with a box exists under abnormal conditions, which was moreover indicated by the dropping of many leaves, the experiment was repeated and conducted in a different manner.

Of 50 leaves, the one-half along the mid-rib was removed and investigated.

The other half with the mesophyll and leaf-stalk was carefully wrapped in tin foil, thus absolutely excluding access of light.

After having been wrapped up for 12 hours or longer, the second half of the leaf was tested with the following result:

I.

C. succirubra leaves.

a. 1st half removed at 6 p. m. Total alkaloid:
0.197 gram in 50 half leaves.

b. 2nd half removed at 6°'clock next morning. Total alkaloid:
0.212 gram in 50 half leaves.

II.

C. Succirubra leaves.

a. 1st half removed at 6. p. m. Total alkaloid:
0.248 gram in 50 half leaves.

b. 2nd half removed at 6°'clock next morning. Total alkaloid:
0.254 gram in 50 half leaves.

III.

C. Succirubra leaves.

a. 1st half removed at 6 p. m. Total alkaloid:
0.233 gram in 50 half leaves.

b. 2nd half removed at 6°'clock next morning. Total alkaloid:
0.207 gram in 50 half leaves.

This experiment was also made in a reverse sense; the entire leaf was first wrapped in tin foil for 14 days and then the first half of the leaf was removed along the mid-rib and tested; the 2nd half was then exposed to the light for 14 days and also tested without mid-rib.

IV.

C. succirubra leaves.

a. 1st -half, after the entire leaf had been wrapped in tin foil for 14 days. Total alkaloid 0.213 gram in 50 half leaves.

b. 2nd half after the same had been again exposed to the light for 14 days. Total alkaloid: 0.198 gram in 50 half leaves.

Now, if alkaloids had formed in the leaf by the assimilation process, these values should have been reversed.

From these investigations it is now evident that when the plant is excluded from the light for ten days (or even a month) this has *no influence* on the alkaloid content of the leaf, whereas Lotsy thought he could even notice a change *after 12 hours.*

The conclusion arrived at by Lotsy that the alkaloid in the Cinchonas is a product of *assimilation* is also incorrect.

If this theory were correct, none, or but very little alkaloid should occur in the leaves which have been excluded from the light for a considerable time, or in fallen leaves; in the latter case the alkaloid

ought to have been transported entirely, or for the greater part, before the falling of the leaves. The investigations show, however, that the mesophyll of plucked, still living, green leaves contains as much amorphous alkaloid as the mesophyll of fallen, brown, no longer living leaves.

It is, therefore, obvious that the alkaloids are products of metabolism which are formed in the leaf or in other organs and remain accumulated there without being of importance, in ordinary circumstances, for the metabolic change.

In order to test the correctness of LOTSY's thesis (l. c. p. 18): "The alkaloid disappearing from the *succirubra* leaf is transported to the stem", the following additional experiments were made.

Twenty well-developed branches of *C. Ledgeriana* were decorticated, that is to say, a strip of bark 4 c.m. in width was removed from the branches and submitted to analysis. The part laid bare was well cleaned to remove all the cambium so that a fresh formation of bark was impossible on that spot.

Eighteen days after the decortication the branch was sawed off at the stem and another 3 c.m. wide strip of bark was removed from both below and above the decorticated piece.

As a new tissue (callus) was beginning to form at the injured surface a strip of a few m.m. wide was left between the injured surface and the new sample, so as to avoid the influence of fresh tissue.

For, it might be possible that the alkaloid content in this new abnormal tissue was also not normal.

1st Experiment.

a. Analysis of the first circular strips of bark tested at once after removal.

7.20 % of quinine, or in 10 pieces of absolutely dry bark 1.69 gram of quinine (alkaloid).

b. Analysis of the sample of bark situated below the circular strip and tested about 14 days after the decortication.

7.35 % of quinine, or in 10 pieces of absolutely dry bark 1,69 gram of quinine (alkaloid).

c. Analysis of the sample of bark situated above the circular strip and tested 14 days after the decortication during which a supply of alkaloid from the leaves might have taken place.

6.60 % of quinine, or in 10 pieces of absolutely dry bark 2.89 grams of quinine (alkaloid).

2nd Experiment,

a. Analysis of the first circular strips of bark tested at once.

8.66 % of quinine, or in 10 pieces of absolutely dry bark 2.89 gram of quinine (alkaloid).

b. Analysis of the sample of bark situated below the circular strip and tested about a month after the decortication.

9.01 % quinine, or in 10 pieces of absolutely dry bark 3.17 grams of quinine (alkaloid).

c. Analysis of the sample of bark situated above the circular strip and tested about a month after the decortication, during which time a migration of alkaloid from the leaves might take place.

7,53 % of quinine, or in 10 pieces of absolutely dry bark 2.79 grams of quinine (alkaloid).

The difference in quinine content, in both experiments, of the samples of bark above and below the ring must be attributed to age, for the bark of the pieces below the decorticated piece is older than that of the piece situated above it, nearer the top of the branch.

The ring test was also applied in a different manner as follows.

A tree about 20 years old with perfectly sound bark was decorticated by removing a strip of bark $13^1/_2$ c. m. in width at the height of a man's chest.

The wood deprived of bark was well scraped thus removing not only the candium but also a part of the young wood, which totally excluded the formation of renewed bark.

At the same time a strip of bark 47 c. m. in length and 2 c. m. in width was removed from just above the decorticated part and also a similar strip from below the same.

First 14 days, then 6 weeks, and finally 3 months after the decortication a second strip next to the first was removed from above and from below the decorticated part and all the four strips were examined.

The result obtained was as follows:

a. Weight of ring bark 236 grams (moist) = 89 grams (air—dry).

Content 7.53 % of quinine (alkaloid) or 2.30 grams of quinine in a strip of bark weighing 34 grams.

b^1. Weight of strip of bark from above the ring 88 grams (moist) = 34 grams (air—dry).

Content 8.10 % of quinine (alkaloid) in the absolutely dry bark or 2.40 grams of quinine in a strip of bark weighing 34 grams.

b^2. Weight of strip bark from above the ring taken 14 days after the first sample.

90 grams moist = 34 grams air—dry.

Content 8.20 % of quinine (alkaloid) in absolutely dry or 2.55 grams of quinine in a strip of bark weighing 34 grams.

b^3. Weight of strip of bark from above the ring taken 6 weeks after the first sample.

106 grams moist = 34 grams air-dry.

Content 7.85 % quinine (alkaloid) in absolutely dry bark or 142 gram of quinine in a strip of bark weighing 34 grams.

b^4. Weight of strip of bark from above the ring taken 3 months after the first sample 104 grams moist = 41 1/7 grams air-dry.

Content 6.67 % quinine (alkaloid) in absolutely dry bark or 1.98 grams of quinine in a strip of bark weighing 34 grams.

c_1. Weight of strip of bark from above the ring tested at once.

90 grams moist = 34 1/2 grams air-dry.

Content 8.40 % quinine (alkaloid) in absolutely dry bark or 2.61 grams of quinine in a strip of bark weighing 34 grams.

c_2. Weight of the strip of bark taken 14 days after the first sample.

90 grams moist = 34 grams air-dry.

Content 8.23 % of quinine (alkaloid) in absolutely dry bark or 2.60 grams of quinine in a strip of bark weighing 34 grams.

c_3. Weight of the strip of bark from below the ring taken 6 weeks after the first sample.

105 grams moist = 34 grams air-dry.

Content 8.54 % quinine (alkaloid) in absolutely dry bark or 2.60 grams in a strip of bark weighing 34 grams.

c_4. Weight of strip of bark from below the ring taken 3 months after the first sample.

92 grams moist = 30 grams air-dry.

Content 9.09 % of quinine (alkaloid) in absolutely dry bark or 2.70 grams of quinine in a strip of bark weighing 34 grams.

From these investigations it is obvious that there can be no question of a migration of alkaloid from the leaves towards the stem; in fact we notice that in the strips of bark from above the ring (see b_2 and b_4) a decrease in quinine has taken place from 7.85 %, six weeks after the decortication to 6.67 % after 3 months of the same or, from 2.42 grams of quinine to 1.98 grams in a strip of bark weighing 34 grams, respectively.

No explanation has been found as yet why the decrease of quinine takes place just in the strips of bark from above the ring while the strips from below the ring show hardly any differences.

One would feel inclined to attribute this to the influence of the

irritation caused by the inflicted wound, although the tree kept to the last fairly healthy as regards the leaves, but in every case it appears that this is out of the question.

In N°. 10 of J. E. DE VRIJ's Kinological studies it is stated that BEHRENS did not succeed in obtaining a trace of a crystalline herapathite when adding a solution of iodine in potassium iodide to the colourless filtrate obtained in due course from the leaf powder.

In order to ascertain whether any crystalline alkaloids are present in leaf mesophyll, the following *modus operandi* was employed.

100 grams of *C. succirubra* leaf deprived of leaf stalk and mid-rib were dried and treated in the manner described. The acid solution finally obtained was again purified by adding alkali and shaking with ether; the ether was then evaporated and the residue dissolved in water, sulphuric acid being added to acid reaction.

The filtrate was evaporated on the waterbath to a syrupy consistency and then dissolved in alcohol.

The alcoholic filtrate was evaporated and the residue dissolved in water and again filtered.

The aqueous fairly colourless solution was rendered alkaline and shaken with ether; the ether was evaporated and the residue subjected to sublimation.

The very small sublimate was taken up with a trace of HCl-containing water, evaporated to dryness in a desiccator and the residue dissolved in a drop of water and filtered.

On heating this filtrate with a trace of a strong solution of sodium hydrogen carbonate, a crystal of chinchonine was obtained.

The presence of a crystalline alkaloid in the mesophyll could also be shown in another, indirect manner.

Attacks of cinchonas by Atlas caterpillars are not rare and as they principally feed on the mesophyll and the leaf stalk and leave the mid-rib untouched both the contents of the stomach and the excreta[1]) of the Atlas caterpillars were examined chemically and in each case cinchonine could be detected but no other crystalline alkaloid.

In the analysis of leaf stalk and mid-rib cinchonidine could be detected as well as chinchonine and judging by the fluorescence, quinine was also present.

In order to compare the results obtained in the analysis of Cinchona, I think I ought to mention briefly the results of the investi-

[1]) When starting from 200 grams of excreta, the other crystalline alkaloids could also be detected.

gation of *Datura strammonium*[1]) by JULIUS FELDHAUS, and of tea by DU PASQUIER[2]) and TH. WEEVERS[3]).

On p. 88, FELDHAUS arrives at the following result in his investigation of *Datura strammonium*.

Blätter: Die Zeit der Einsammlung ist ohne Einflusz auf den Alkaloidgehalt, denn in einem Falle enthielten die Ende Juli und die Ende August und in einem anderen Falle die Anfang September und Anfang Oktober gesammelten Blätter derselben Pflanzen eine nicht wesentlich verschiedene Menge Alkaloid. 0,46% respektive 0,46% Alkaloid und 0,30% respektive 0,39% Alkaloid.

Der Gehalt junger, an der Basis noch gelbgefärbter, etwa 5—10 cm. langer Blättchen mit 0,48% Alkaloid war nicht wesentlich verschieden von dem vollentwickelter, zu gleicher Zeit von denselben Pflanzen gesammelter Blätter, der 0,49% Alkaloid betrug. Damit ist die Ansicht SÜM-JENSENS, zu der er auf Grund seiner mikrochemischen Betrachtungen bei Hyoscyamus gelangt war, nicht bestätigt, dass in jungen Blättern der Alkaloidgehalt relativ grösser zu sein scheine

Die weiteren mikrochemischen Untersuchungen von SÜM-JENSEN sowohl die von PH. MOLLE zeigten, dass die grösste Alkaloidmenge in den Gefässbündeln, wenig oder gar nicht im Mesophyll der Blätter zu finden sei.

Ich fand im Assimilationsgewebe 0,48%, in Mittel- und Sekundärnerven 1,39% und in den Blattstielen derselben Blätter 0,69% Alkaloid.

Bei Hyoscyamusblättern hatte E. SCHMIDT eine Trennung in Blattflächen und Blattstiele vorgenommen und fand in den Blattflächen 1) 0,2726% und 2) 0,2861% Alkaloid, in den Blattstielen 1) 0,36% und 2) 0,365% Alkaloid. Also auch bei Hyoscyamus ein höherer Gehalt an Alkaloid in den Blattstielen als in den Blattflächen.

Eine ergiebige Chilisalpeterdüngung ist ohne Einfluss auf den Alkaloidgehalt. Blätter von Pflanzen, die auf ungedüngtem Beete gewachsen waren, hatten 0,49% Alkaloid, von Pflanzen, die auf dem Salpeterbeete gewachsen waren, 0,50% Alkaloid. Samen von Pflanzen der ersten Sorte hatten 0,34% Alkaloid, von der zweiten Sorte 0,34% Alkaloid.

Assimilation. Verdunkelung ist auch von keinem Einflusse auf den

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[1]) Quantitative Untersuchung der Verteilung des Alkaloides in den Organen von Datura stramonium. Inaugural Dissertation von JULIUS FELDHAUS, Marburg 1903.

[2]) Beiträge zur Kenntnis des Thees. Inaugural Dissertation von PAUL A. DU PASQUIER, Zürich 1908.

[3]) Die Physiologische Bedeutung des Koffeins und des Theobromins von TH. WEEVERS. Annales du Jardin Botanique de Buitenzorg (Volume XXI) 2e Serie. (Volume VI) 1e Partie.

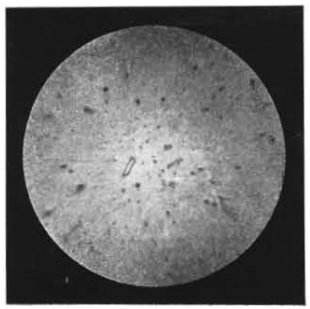

Fig. I.

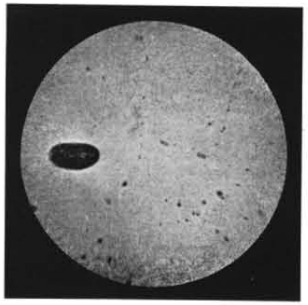

Fig. II.

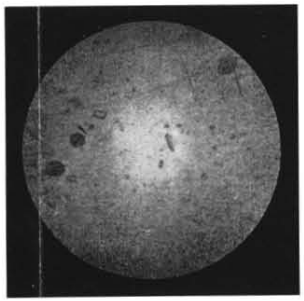

Fig. III.

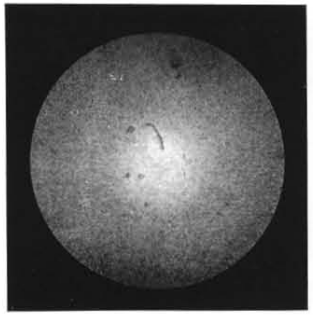

Fig. IV.

Fig. V.

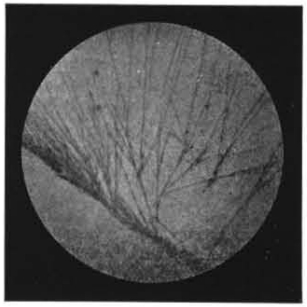

Fig. VI.

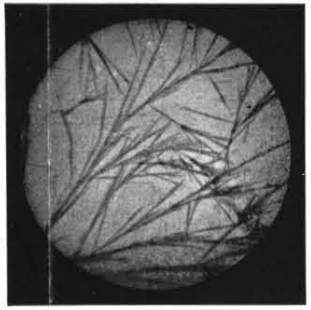

Fig. VII.

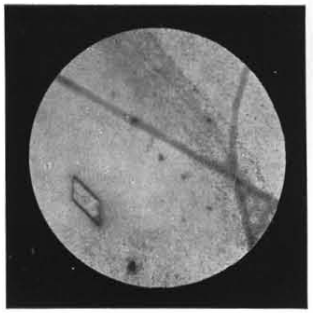

Fig. VIII.

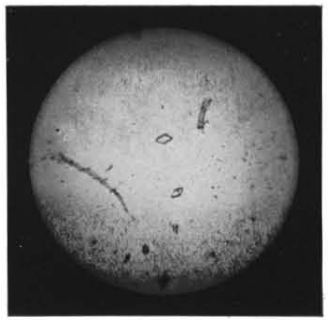

Fig. IX.

Fig. X.

Alkaloidgehalt. Im Dunkeln aufgewachsene Keimpflanzen hatten, 0,66% Alkaloid, normal aufgewachsene derselben Samen 0,67% Alkaloid.

Ebenso konnte CLAUTRIAU keinen Unterschied im Alkaloidgehalte hell und dunkel erwachsener Keimpflänzchen von Coffea- und Thea-Arten beobachten.

Blatthälften am Abend gesammelt hatten 0,48% Alkaloid, die zugehörigen Blatthälften am folgenden Morgen gesammelt hatten 0,40% Alkaloid. Blatthälften abends gesammelt hatten 0,51% Alkaloid, die zugehörigen Blatthälften, nach dreitägiger Verdunkelung gesammelt, hatten 0,51% Alkaloid. Es findet also während der Nacht oder künstlicher Verdunkelung keine Ableitung des Alkaloides statt.

Es tritt aber auch bei Tage keine wesentliche Vermehrung des Alkaloidgehaltes in ausgewachsenen Blättern ein, ich müsste sonst, da ja keine Ableitung stattfindet, in den an verschiedenen Tagen gesammelten Blatthälften derselben Blätter einen wesentlich höheren Alkaloidgehalt in den später gesammelten Hälften gefunden haben. Ich fand in Blatthälften 0,33% Alkaloid, in den zugehörigen, nach drei Tagen ohne künstliche Verdunkelung gesammelten Blatthälften fand ich 0,33% Alkaloid.

Die Verletzung des Blattes veranlasste also auch nicht eine stärkere Alkaloidproduktion.

Aus allen Versuchen geht hervor, dass das Alkaloid kein direktes Produkt der Wirkung des Lichtes auf die Blätter ist, also auch kein Assimilationsprodukt.

On pg. 36, DU PASQUIER arrives at the following result in his investigation of tea.

"Alle drei Wege führten mithin zum selben Resultate: Koffein spielt in der Theeflanze die Rolle eines Abfallproduktes."

DU PASQUIER found in 50 fallen tea leaves (weighing when dry 11.000 grams) a total weight of 0.1001 gram of caffeine = 0.91%.

On pg. 12 he further states:

Vergleicht man diese Zahlen mit meiner früheren Reihe (Seite 21, Tabelle VIII), so sieht man, dass sie sich aufs schönste an jene Zahlen angliedern würden, sodass also ein Rückgang oder sogar ein Verschwinden im Koffeingehalt bei den abgefallenen Blättern nicht zu erkennen ist.

This result, however, does not agree with that of WEEVERS, who does not find any caffeine in the fallen tea leaves.

In regard to this DU PASQUIER offers the following explanation:

Es war mir denn auch nicht schwierig, die Erklärung für das Nichtauffinden von Koffein durch WEEVERS—DE GRAAF zu geben.

Dieselben verwendeten zur Koffeinbestimmung eine Methode, in der die Blätter mit ungelöschtem Kalk behandelt werden. Nun hat aber A. BEITTER 1901 in seiner Arbeit "Neuere Erfahrungen über Koffeinbestimmung" nachgewiesen, dass beim Behandeln mit Kalk die Hälfte des ganzen Koffeins zersetzt wird. Bedenkt man ferner, dass BEITTER seine Beobachtungen an mehrere Prozent Koffein enthaltenden Thees machte und dass der Prozentgehalt der abgefallenen Blätter an Koffein nicht einmal 1 % beträgt, so wird man leicht einsehen können, dass die geringe Menge Koffein leicht übersehen wurde, und WEEVERS Begründung mithin nicht stichhaltig ist. Dazu kommt, dass sie keine quantitativen Bestimmungen machten, sondern nur den qualitativen Nachweis zu führen suchten.

By way of comparison the leaves of a tea shrub were also investigated. The results obtained agree with those of DU PASQUIER in so far that caffeine could be plainly detected in the leaves of a tea shrub which had been excluded from the light for 14 days.

Owing to the want of material no investigation could be made as to the presence of caffeine in the fallen leaves.

I wish to give my best thanks to Dr. A. RANT, botanist at the Government Cinchona exploitation for his suggestions and advice in this investigation.

CONCLUSIONS.

The conclusions arrived at in this research are as follows:

1. The contention of J. P. LOTSY that an exposure of the leaf to light or darkness affects the alkaloid content is incorrect.
2. His view that the formation and migration of the alkaloid is affected by the weather is also incorrect.
3. The alkaloid is not an assimilation but a metabolic product.
4. The mesophyll and the veins of both *C. Ledgeriana* and *C. succirubra* leaves contain most decidedly crystalline alkaloids and also quinine.
5. The leaf stalk and the mid-rib of *C. Ledgeriana* and *C. succirubra* contain besides cinchonine also quinine.

EXPLANATION OF THE ILLUSTRATIONS.

Fig. I. Cinchonine from leaves of C. succirubra.
Fig. II. Cinchonine crystals obtained with sodium hydrogen carbonate from the excreta of the Atlas-caterpillar.
Fig. III. Cinchonine crystals obtained with sodium hydrogen carbonate from the contents of the Atlas-caterpillar.

Fig. IV. Cinchonine crystals obtained with sodium hydrogen carbonate from the chrysalis of the Atlas-caterpillar.
Fig. V. Sublimate of theïne recrystallised from water (dark).
Fig. VI. Sublimate of theïne recrystallised from water (light).
Fig. VII. Theïne (dark) with sodium acetate.
Fig VIII. Cinchonine from mid-rib and leaf stalk.
Fig. IX. Cinchonine from mesophyll and veins.
Fig. X. Cinchonidine from mid-rib and leaf stalk.

Physiology. — *"The temperature optimum of physiological processes"*. By Miss J. VAN AMSTEL and Prof. G. VAN ITERSON Jr. (Communicated by Prof. M. W. BEIJERINCK).

(Communicated in the meeting of May 28, 1910).

The destruction at high temperature of the active principle, so conspicuous in physiological processes, has been subjected by TAMMANN [1]) for a few enzyme processes to a nearer research, which led that investigator to the result, that destruction of the enzyme by heating takes place after the equation of monomolecular chemical reactions. In accordance with this view the relation between the quantity of enzyme y, which after heating at a constant temperature during a time t, is still active, and that time, would be represented by the formula: $k = \frac{1}{t} log \frac{a}{y}$, where a represents the originally present quantity of enzyme and k a constant.

DUCLAUX [2]) suggested a relation between the destruction of the active agency by high temperature and the optimum of enzyme action, and explained the occurrence of this cardinal point by admitting that the velocity of the reaction continually increases with the rising of the temperature, whilst the bending of the curve, which represents the relation between velocity and temperature, should exclusively be ascribed to a steadily increasing destruction of the enzyme by the heating. The views of DUCLAUX were absolutely theoretical and he made no experiments to test them.

The idea which forms the base of DUCLAUX' theory we find back in a treatise of BLACKMAN [3]), but here the views put forward à priori are tested by observations and in particular by the results of studies made conjointly by this investigator and Miss MATTHAEI [4]) on the relation of the carbonic acid assimilation with the temperature.

[1]) Zur Wirkung ungeförmter Fermente, Zif. f. Physikal. Chem., Bd. 18, 1895, S. 429.
[2]) Traité de Microbiologie, T. II, 1899, p. 193.
[3]) Optima and Limiting Factors, Annals of Botany, Vol. XIX, 1905, p. 281
[4]) Phil. Trans. Roy. Soc., Vol 197 B, 1904, p. 85.