PHILOSOPHICAL TRANSACTIONS.

I. *Chemical and Physiological Experiments on living Cinchonæ.*

By J. Broughton, *B.Sc., F.C.S., Chemist to the Cinchona-Plantations of the Madras Government. Communicated by Dr.* E. Frankland, *F.R.S.*

Received May 16,—Read June 16, 1870.

On the Neilgherry Mountains in South India are now growing nearly three millions of trees of cinchona of various species. The greater part of these are on plantations belonging to Government, and are the result of the introduction from South America and successful naturalization of these valuable febrifuge-yielding plants by the Government of India, under circumstances which have long since been made public.

The chemical investigations which during the last three years have been made, for the purpose of settling the various economic questions connected with the production of the febrifuge constituents of the bark, have led to some conclusions of scientific interest. I have the honour in the subsequent pages of communicating to the Royal Society the most important of these, and the experimental grounds on which they depend. These inquiries have been made under circumstances of great advantage, for the living plants have never before been under the control of the experimenter. The ability to study the changes occurring in the growing tissues cannot fail to throw light on the formation and physiological functions of the chemical constituents whose production is the object of the undertaking.

The organic principles which characterize these cinchona-barks are the alkaloids Quinine, Cinchonidine, Cinchonine, and occasionally Quinidine, the peculiar bitter principle Quinovin, the acids Quinic and Quinotannic, and in small amount another not fully investigated.

Without attempting to describe the well-known alkaloids, it may be stated that all the facts known point to a marked natural connexion between quinine and cinchonidine, notwithstanding the difference of an atom of oxygen in their composition. Thus the analysis of the individual plants will frequently give results which show the same amount of alkaloids, but which differ by the respective *quantities* of these alkaloids, while all

other constituents remain unaffected. Chemically these alkaloids greatly resemble each other, their solutions both rotate the plane of polarization to the left, and their salts and behaviour with reagents are very similar. With the exception that cinchonidine will crystallize readily from alcohol, and produces no green colour with chlorine and ammonia, their reactions differ in degree only*. The circumstances under which they mutually occur in living cinchona-barks, taken in connexion with their chemical properties, would lead me to class the alkaloids in two groups: 1st. Quinine, Cinchonidine, Quinidine; 2nd. Cinchonine. I have never yet met with the alkaloid aricine in any Indian cinchona-bark, though it has frequently been looked for.

Quinovin is a very constant constituent of all the parts of a cinchona-plant, as is also quinic acid; the latter is the acid of cinchona, as tartaric acid is that of the grape, and is found in the free state in most of the juices. The peculiar red substance known as cinchona-red, though found in all dried bark, does not exist in the living plant, and is formed by the action of the free oxygen upon the peculiar tannin: hence a slice of fresh bark always becomes brownish red when the internal tissues are exposed.

On account of their being the most vigorous, the oldest, and in suitable climates the hardiest trees, I have generally chosen the plants of *C. succirubra* for the subjects of experiment in cases where a special treatment of the plant was necessary. The bark of this kind is very rich in alkaloids and the cinchona tannin: it may contain all the alkaloids, though quinidine is rarely found; the other three invariably occur. It has a great tendency to produce cinchonidine. I have every reason for believing that the natural processes resulting in the formation of the several alkaloids are similar throughout the quinine-yielding species though differing in degree.

Occurrence of Alkaloids in various parts of the Plant.

The bark is *par excellence* the seat of the alkaloids, that of the trunk being the richest. I here quote analyses of the trunk-bark of various species growing on the Neilgherries, showing the percentages of alkaloids in the dry bark.

	1.	2.	3.	4.	5.	6.	7.
	A lanceolate-species tree, name unknown, 5 years.	*C. officinalis*, 5 years old, fine tree.	*C. officinalis*, 5 years old, mean tree.	*C. Calisaya*, 2½ years, mean tree.	*C. succirubra*, 7 years old, mean tree.	*C. peruviana*, 5 years old, mean tree.	*C. micrantha*, 4 years old, mean tree.
Total alkaloids	11·40	6·76	4·34	4·53	7·43	6·25	7·1
Quinine	9·75	3·76	2·18	3·27	1·72	0·41	0·3
Cinchonidine and cinchonine	1·65	3·00	2·16	1·26	5·71	5·84	6·8
Crystallized cinchonidine sulphate obtained	0·38	1·94	2·00	0·70	4·92	1·80	0·0
Ditto quinine sulphate	10·13	3·45	1·60	2·17	1·51	0·00	0·0
Cinchonine	......	......	......	......	......	3·84	6·8

* HOWARD (Illustrations to the 'Nuova Quinologia' of Pavon) states that the cinchonidine found in *C. peruviana* is that peculiar cinchonidine described by WITTSTEIN (J. pr. Chem. 72, 101), and generally known as

No. 1 refers to a kind (whether species or variety is not accurately determined) which was found on the plantation among some plants raised from seed brought by Mr. Cross from the Loja district; it gives the largest yield of quinine of any kind known. The first five are quinine-yielding kinds, but in which cinchonidine may occur in greater or less degree. Nos. 6 and 7 are nearly destitute of quinine.

The thin bark on the larger roots is still richer in alkaloids, and in *C. succirubra* the yield is generally about 9·70, and may reach 12 per cent. It consists principally of cinchonidine and cinchonine. The root-bark of *C. officinalis* is also rich in alkaloids; analysis gave 5·4 per cent. The root-bark has been little investigated, as the difficulty of obtaining it would render it an unsuitable source of alkaloids. I ascribe its richness in alkaloids, for reasons given below, to the circumstance of it being shielded from the rays of the sun.

The leaves of cinchona are bitter from the presence of quinovin, and acid from free quinic acid. In many early trials I failed to get full and satisfactory evidence of the presence of alkaloids. By working with great care upon 20 lbs. of the leaves, I obtained a small amount of rough uncrystallizable alkaloid, which, by solution in weak acetic acid, exposure to a stream of air to facilitate oxidation, decolorization with animal charcoal, reprecipitation, drying, several times repeated, I procured tolerably pure and colourless. From this I obtained well-defined crystals of the quinine sulphate and oxalate, and unmistakable crystals of cinchonidine and cinchonine from their weak alcoholic solutions: the amounts are, however, very small. Other trials, conducted quantitatively, gave the following results, which are given in percentages of the dry and fresh leaves:—

	C. succirubra.		*C. officinalis.*	
	In fresh.	In dry.	In fresh.	In dry.
Total alkaloids	0·0041	0·019	0·0035	0·0111
Quinine	0·0016	0·008	0·0015	0·005
Cinchonidine and cinchonine	0·0025	0·011	0·0020	0·006
Quinine sulphate obtained in crystals	0·00078	0·0037	0·0008	0·002

It is evident from the above that the leaves are useless as a source of alkaloids. The state of their occurrence is wholly different to that in which they occur in the bark.

The wood of cinchona also contains the alkaloids in small amount; they are there associated with a red resin and the cinchona tannin. In *C. succirubra* the alkaloids may amount to 0·1 per cent., of which 0·03 is quinine and the remainder cinchonine and cin-

Wittstein's cinchonidine. In my examination of the bark of *C. peruviana*, I also found a form of cinchonidine the pearly centre of whose crystalline sulphate and other properties agreed exactly with Wittstein's description. By repeated crystallization of its sulphate from water, and then of the free base from alcohol, it was obtained apparently pure; it was then carefully compared with a standard specimen of ordinary cinchonidine in the polarimeter, and found to possess precisely the same rotatory power. I cannot therefore consider it a distinct substance; I therefore only recognize one form of cinchonidine in the text.

chonidine. The wood of *C. officinalis* contains much less. All the several alkaloids have been satisfactorily identified in the bases obtained from wood, by being obtained in well-defined microscopic crystals yielding in solution their respective reactions.

The beautiful scented blossoms contain no alkaloids, but are rich in quinovin and quinic acid.

The fruit also contains no alkaloids. It is very sour from the presence of quinic acid; it is a most convenient source of this acid. The juice of the fruit mixed with milk of lime and filtered, readily yields on evaporation an abundant crop of crystals of calcic quinate.

The ripe seed contains but traces of the characteristic principles of cinchona and no alkaloids.

The milky fluid which fills the laticiferous vessels cannot be obtained in sufficient quantity for analysis. It is not bitter, and contains in all probability little or no alkaloid. These vessels are rarely contained in the *trunk*-bark of *C. succirubra*, and can therefore scarcely be supposed to be concerned (directly at least) in the production of alkaloids.

State of combination in which the Alkaloids exist in the Bark.

This, after many trials, was determined by the following method. A quantity of the best trunk-bark of *C. succirubra* was exposed to strong pressure in a very powerful screw-press, by which means the juices were pressed out so perfectly that the bark was apparently almost dry; and subsequent examination under the microscope showed that the parenchymatous cells were quite broken up, and many of the liber-cells also.

The juice at first obtained was of a greenish-yellow colour; it, however, absorbed oxygen rapidly and became red. When first obtained, it was very turbid from the presence of much alkaloid tannate of a grey colour, rapidly becoming brownish red; this soon subsided in a well-corked flask, and left the liquid clear. The supernatant liquid was bitter and strongly acid from free quinic acid; it also contained free tannin. Its specific gravity was 1·034 at 17° C.

It was now assumed that the whole of the moisture lost by the bark on drying over oil of vitriol represented the water of this juice; this assumption, if not entirely true, will not differ sufficiently from the truth to seriously affect the subsequent results. The water of hydration of the woody fibre &c. parted with under the circumstances would obviously be a very small portion of the whole; hence the specific gravity of the juice being known, the whole weight of juice present in the bark is also known.

If, now, the whole amount of alkaloid present in the bark is determined, and also that contained in solution in the juice, the difference will express the amount of alkaloids existing in an insoluble form in the living bark.

The analytical data are as follow:—

Moisture lost by bark on sulphuric acid 67·5 per cent.

	In fresh bark.	In 100 parts of juice.
Total alkaloids	6·10	1·17
Quinine	1·15	0·31
Cinchonidine	3·65	0·51
Cinchonine........................	1·30	0·35

whence, by an obvious calculation, in the sample of fresh bark the respective alkaloids existed as follows:—

	In natural solution.	In an insoluble state.	Total in bark.
Total alkaloids	0·82	5·28	6·10
Quinine	0·22	0·93	1·15
Cinchonidine	0·35	3·30	3·65
Cinchonine....................	0·24	1·06	1·30

It thus appears that the greater part of the alkaloids is contained in the bark in the solid state; none can be in the free state, on account of the excess of quinic acid and cinchona tannin. Hence it must be concluded that six-sevenths of the alkaloids exist in the cells of this bark as insoluble tannates, the remaining seventh mainly existing in solution as quinate, though the solution is of course saturated with tannate in addition.

These combinations of the alkaloids with tannin are very sparingly soluble in cold water. The whole state of things in the bark resembles the equilibrium of solution and precipitate which would result from a mixture of tannin, alkaloid, and quinic acid. The quinic acid is only able to decompose a small portion of tannate, and the tannin is not able to precipitate the whole alkaloid from solution. Actual trial with the respective substances produces these effects. It is to be remarked that there is no marked difference in the proportions of the respective alkaloids which exist, in one or the other state, in the cell-contents or in the liquid which moistens the whole. This is in harmony with the impression, to which all the facts at present known seem to conduce, that all the cinchona alkaloids are physiologically equivalent.

From the nature of the weak compound that quinovin is able to form with the cinchona alkaloids, it is evident that none could exist in the presence of quinic acid. Nearly the whole of the quinovin is to be found in the bark after the juice has been squeezed out. The clear juice only contains a very small amount, and its presence is readily explained by the faint solubility of this substance in water.

The whole of the quinovin exists therefore in the insoluble and free state.

No thoroughly accurate means of determining the amount of the cinchona tannin has yet been devised; neither gelatin, animal membrane, nor even plumbic acetate removes it completely from solution. Tartar emetic succeeds better; but the precipitate has a variable composition, and also removes the alkaloids in part. By washing these out

from the precipitated compound of oxide of antimony and tannin formed in an acetic-acid solution, and subsequently determining the amount of antimony in the precipitate, I estimated the amount of the peculiar tannin in the juice at 2·35 per cent. No verification, however, could be made, as it is scarcely possible to procure this peculiar tannin in a pure state, from its excessive proneness to oxidation.

The tannates of the alkaloids appear quite similar, when prepared artificially, to those found in the bark. When obtained as mentioned above from the barks, they are readily decomposed by mineral acids. They oxidize very rapidly, and form red substances, which, when moist, are decomposed with moderate ease by acids with separation of cinchona-red, and very readily, but not quite completely, by alkalies, which dissolve the cinchona-red and leave the alkaloids in a coloured state. If the tannates are heated to 100° and become quite dry, they occasionally form a red compound, which resists the action of dilute mineral acids even on boiling. This is a fruitful cause of inaccurate bark analysis, when the bark has been long dried in a water-bath previously. In such cases it is better to treat the bark with alcoholic potash, as recommended by DE VRY (Pharm. S. Trans. vol. vi. p. 50).

Order in which the Alkaloids are formed in the living Tissues of the Bark.

Very much light has been obtained on the order in which the natural formation of the cinchona alkaloids proceeds, by making periodic examinations of rapidly forming bark-tissues.

When cinchona-bark, not exposed to the direct rays of the sun, is carefully removed in strips without injury to the delicate cambium-cells underneath, such bark is rapidly renewed by natural processes. The cambium immediately thickens, so that after a little more than a week a thin cellular layer of a light green colour has covered the wound. On the surface of this, light brown granulations are found which spread and thicken. For many months only parenchymatous cells are to be seen; but subsequently short woody cells are found, which lengthen and increase in number and thus form a liber. The so-called resin-cells do not appear, nor have I observed any laticiferous vessels in this renewed bark. A true cork is, however, formed.

Bark thus rapidly renewing has been analyzed at various intervals of the process. The following series is one of many; but I quote it here because it possesses an additional element of precision in being a set of experiments performed on the trunk-bark of the same tree of *C. succirubra*.

	After four months.	After six months.	After ten months.	After fifteen months.	After seventeen months.
Total alkaloids	4·30	5·30	5·03	6·00	5·47
Quinine	4·20	4·40	4·02	3·03	2·65
Cinchonidine and cinchonine	0·10	0·90	1·01	2·97	1·82
Crystallized sulphate quinine obtained	0·25	1·56	2·20	2·30	3·12

These analyses lead to some conclusions of importance. The alkaloid first found in the freshly forming tissues is *quinine*, although the tree is of a kind producing mainly cinchonidine. As first formed, however, the quinine refuses to form crystalline sulphates; it is "uncrystallizable quinine." After two months more growth a third part of this quinine has obtained the ability to form crystallizable salts, and a small amount of cinchonidine and cinchonine are formed, both of which are recognized by their crystalline form under the microscope. These latter constantly increase; and in the above and other similar experiments the increase seems made at the expense of the crystallizable quinine, which simultaneously diminishes. The order of formation in point of time is thus shown to be, 1st, uncrystallizable quinine; 2nd, crystallizable quinine; 3rd, cinchonidine and cinchonine.

Furthermore, the very local character of these peculiar changes seems to render it highly probable that the alkaloids are really formed *in situ* in the very tissues in which they are found,—that is, the very cells which contain them also have formed them. It has been shown above how very minute an amount of alkaloids the leaves, wood, and twigs contain. The quinine in the leaves, small though it be, forms a crystalline sulphate. The alkaloid that is found in the freshly forming bark refuses to crystallize; it is therefore scarcely credible that it owes its origin to the distant leaves (according to the prevalent notion that all the plant principles are formed therein). If this could be conceived to be the case, it would at least be expected that the crystalline principles would appear first. Also for seventeen months after its formation the newly made bark is found to differ in composition from the rest of the bark of the tree by containing much quinine and little cinchonidine, precisely the reverse to what is contained in the older portions. The conclusion thus appears to be inevitable that the alkaloids are formed *in situ*. I find that Mr. HOWARD* on other grounds also inclines to the same conclusion.

The opinions as to the actual seat of the alkaloids in cinchona-bark have been divided. WADDELL, WIGAND, and others have stated on deductive grounds that they are mainly contained in the liber; HOWARD, on the other hand, by several direct experiments made with various South-American barks, found the largest amount in the external cellular portions ('Nuova Quinologia' of Pavon, Appendix). By adopting HOWARD's method of actually separating the liber, and making an analysis both of it and the remaining cellular portion, I was enabled abundantly to corroborate his results. The following analyses illustrate mine:—

	1st Series.		2nd Series.	
	Liber.	Cellular portion.	Liber.	Cellular portion.
Total alkaloids	5·94	7·98	6·85	8·00
Quinine	0·70	2·25	0·85	3·25
Cinchonidine and cinchonine	5·24	5·74	6·00	4·75
Sulphate quinine obtained	......	......	0·90	2·80
Sulphate cinchonidine obtained	......	......	4·10	4·20

* Quinology of the East-Indian Plantations, p. 16.

The above was made with young bark whose liber contained parenchymatous as well as woody cells. In older bark the difference would be still more marked. The fact of the quinine being contained mainly in the exterior cellular tissue is in harmony with its occurrence in newly forming structures, as detailed above.

I have never yet succeeded, although many attempts have been made, in converting quinine into cinchonidine, or *vice versâ*. Nevertheless many phenomena in the living plant point to the conclusion that a real change of quinine into cinchonidine occurs therein. The order of the appearance of the alkaloids in renewing bark, the comparative absence of quinine in the highly organized liber, the changes produced by sunshine and heat, all point to such conversion as a legitimate hypothesis; it is one that is not contradicted by a single fact, and is in harmony with observations made with very diverse species of cinchona, and with the chemical similarity in the behaviour and properties of the two substances.

Influence of Sunlight and Heat on growing Cinchona-bark.

PASTEUR* first pointed out that quinine, cinchonine, &c. are converted, under the influence of a high temperature, into the isomeric uncrystallizable alkaloids, quinicine and cinchonicine. He also showed that the same change was effected by exposing the salts of the alkaloids to sunshine. On these grounds he asserted that the plan of drying cinchona-bark in the sunshine, as practised in South America, is injurious to its quality.

Many experiments made during the last two years have amply corroborated these assertions. The purest and whitest alkaloids I have been able to prepare become coloured brown when exposed to the Indian sunshine, and the change is still more rapid when exposed in the form of salts. All the four alkaloids are affected in the same manner, even when sealed *in vacuo*, but the change is more readily effected in the case of the salts of quinine. In the solid state the change is very superficial; and in cases where the uncrystallizable alkaloids are formed more abundantly, the amount produced is only a small percentage of the whole, even when the insolation has lasted weeks. Pure quinine sulphate is especially sensitive to light, but becomes far less so when a small amount of the corresponding salt of cinchonidine is present, as frequently is the case in the commercial salt.

In the course of a long series of experiments made to determine the best practical method of drying the bark for export, the effects of heat and sunshine were abundantly manifest. The change produced by sunshine on the bark requires some time to become very perceptible. It is thoroughly apparent when the drying of the fresh bark is checked by exposing it for a fortnight in a box covered with glass; a decrease in the yield of the crystalline sulphates then becomes clearly evident, and amounts to from 0·8 to 1·1 per cent. of the dried bark. If the bark be finely shred (to increase the surface of action) and exposed for three days to the cloudless Indian sun, the alkaloids also lose the power of crystallization to the amount of 0·6 per cent. In both cases I have never

* Comptes Rend. vol. xxxvii. p. 110.

succeeded in obtaining exactly the same weight of free alkaloids. After exposure the loss varies greatly, from 0·4 to 1 per cent. According to PASTEUR there should be no loss of weight when the crystallizable alkaloids are converted into their isomeric crystalline forms. Direct trials showed that this loss is connected with the effect of sunshine; for when the bark is dried at 50° C., or *in vacuo* over sulphuric acid, no such loss occurs.

No alteration in the relative amounts of quinine and cinchonidine could even be detected as the effect of insolation or artificial heat.

The damaging effect of light is not practically of great importance in the commercial preparation of the bark. The dry air and fierce sun of the Neilgherries with care may be made to dry the green bark so rapidly that sufficient time does not elapse for any appreciable deterioration of quality to be effected; nevertheless the sagacious dictum of PASTEUR must be held to be true.

A high temperature, or even a steam-heat, is prejudicial to the quality of cinchona-bark, for reasons before mentioned. The former converts part or the whole of the crystallizable alkaloids into their uncrystallizable isomers; the latter often causes a compound of cinchona-red to be formed with the alkaloids, which is decomposed with difficulty by acids.

The following series of analyses, though conducted with special reference to economical results and the methods of commercial manufacture, aptly illustrate the foregoing principles. In all cases the finely shred bark was dried by the several methods; the fine division exaggerates the difference of result.

Red Bark.

	Fresh bark.	Dried at 17° C. *in vacuo.*	Dried at steam-heat.	Dried in current of air at 35°–45° C.	Dried in sunshine.	Dried in sunshine slowly.
Total alkaloids	6·89	6·67	5·92	6·64	5·90	5·85
Crystalline sulphates obtained .	6·12	5·98	5·31	5·89	5·28	4·78
Comparative amounts of acid required to neutralize the alkaloids	13·2	13·1	11·8	13·1	12·0	11·6

Mixed Crown Barks.

	Fresh bark.	Dried at 17° C. *in vacuo.*	Dried at steam-heat.	Dried in current of air at 35°–45° C.	Dried in sunshine.	Dried in sunshine slowly.
Total alkaloids	3·93	3·92	3·52	3·85	3·63	3·04
Crystalline sulphates obtained ...	3·67	3·52	3·01	3·56	3·11	2·88
Comparative amounts of acid required	7·2	7·0	6·6	6·8	6·7	5·9

The effects of the sun's rays on cinchona-bark, when separated from the tree, being

thus marked, it is to be expected that their action on the growing bark produces changes of a similar character. Experiment shows that this is the case to a great extent.

A method has been introduced into cinchona-cultivation by Mr. M^C^Ivor, Superintendent of the Government Neilgherry Plantations, which, when applied to young trees, produces some remarkable results. It consists in covering the bark of the trunk with a coating of moss. After the expiration of a year or eighteen months, the bark is found to be greatly improved in quality, to have become rather thicker, and to contain a larger general yield of alkaloids as well as an augmented proportion of quinine. In illustration of this I may quote the following analyses of the barks of trees of *C. succirubra* that have been thus treated:—

	No. 1. Bark of *C. succirubra* sixteen months under moss.	No. 2. Bark of same trees renewed under moss sixteen months.	No. 3. Original bark.
Total alkaloids	10·72	8·22	6·74 per cent.
Quinine	4·31	5·14	2·40 ,,
Cinchonine and cinchonidine	6·41	3·08	4·34 ,,
Total sulphates obtained crystallized	9·27	4·67	6·06 ,,
Sulphate of quinine	4·02	3·87	2·21 ,,
,, cinchonidine	5·25	0·80	3·85 ,,

From certain observations I had made on the difference in the barks of trees grown in shade and sunshine, and the known changes produced by the latter both on the alkaloids and barks, I was led to believe that the singular improvement in quality produced by mossing the bark was the effect of shielding it from the prejudicial action of the sun's rays, whether acting directly or indirectly. In order to test the truth of this by experiment, I covered the trunk-bark of two trees of *C. succirubra*, one with a shield of tin plate, and the other with a double fold of black alpaca cloth. The effect of this would be to keep the bark in darkness, while the access of air was not impeded. After ten months' protection analyses were made, and the following Table expresses the results.

	Tree covered with tin plates.		Tree covered with black cloth.		
	Original bark.	Bark after ten months' protection.	Original bark.	Bark after six months' protection.	Bark after ten months' protection.
Total alkaloids	5·29	8·10	5·04	6·91	7·92
Quinine	2·16	1·65	2·26	2·03	2·34
Cinchonidine and cinchonine	3·13	6·45	2·78	4·88	5·58

The protection from light has thus, in the otherwise dissimilar instances, had the effect of greatly increasing the amount of alkaloids. It is remarkable, however, that the quinine has not shared in the increase, a consequence I was not prepared to find,

and for which I can only partially account. An experiment made in the warmer climate of Wynaad, in which the bark was shielded with thick coarse woollen cloth, produced similar results to the above, in which an increase of quinine was also obtained. Hence it seems demonstrated by these experiments that, even while the bark is growing, there is a constant deterioration and waste going on by the action of the sun's rays, which is prevented by removing the cause. Whether it is the light of the sunshine which produces the effect, or the comparatively high temperature produced in the outer cellular portions where the alkaloids are mainly situated, is a point still to be determined.

A study of the differences in the amounts of the respective alkaloids found in the barks grown at different elevations appears to throw some light on the effects of temperature. A series of trees was chosen of as nearly the same age as possible, and growing on soil of the same character, but growing at very various elevations, and hence under climates of very various annual mean temperatures. The trunk-bark of these trees was collected and analyzed. This was done at a time of the year when the yield of alkaloids is nearly at a minimum; there is, however, no reason for supposing this circumstance would alter the comparative value of the results. The following tabular statements express the results:—

Red Bark.

	I.	II.	III.	IV.	V.	VI.	VII.
	7770 ft.	7660 ft.	7450 ft.	6560 ft.	5450 ft.	3500 ft.	2300 ft.
Total alkaloids	2·38	2·03	4·21	5·75	3·8	4·10	4·1
Quinine	0·45	0·33	0·70	2·50	1·2	0·86	0·6
Cinchonidine and cinchonine	1·93	1·70	3·51	3·25	2·6	3·24	3·5
Crystalline sulphates obtained	1·75	1·60	3·20	4·20	3·1	3·58	3·4

Crown Bark.

	I.	II.	III.	IV.
	8000 ft.	7770 ft.	6007 ft.	5450 ft.
Total alkaloids	3·42	3·61	3·10	2·68
Quinine	2·10	1·83	0·75	0·45
Cinchonidine and cinchonine	1·32	1·78	2·35	2·23
Crystalline sulphates obtained	3·20	3·30	2·60	1·80

The analyses in the first Table were made with the product of trees which, on account of the comparatively cold climate, were stunted and unhealthy in appearance, and thus are scarcely fit subjects for the inquiry. Analyses IV., V., VI., VII. agree in showing that from a certain point an increase of mean temperature has the effect in diminishing the amount of quinine, and to a less marked extent the total alkaloids. The same effect

is also evident in the more hardy *C. officinalis*. Many other analyses of cinchona-bark grown at low elevations have abundantly shown that a high mean temperature is adverse to the production of quinine. These results, taken in conjunction with those obtained from shielded bark, seem to demonstrate that sunlight degrades the alkaloids generally, while heat mainly diminishes the amount of quinine.

It would naturally be expected that, as the barks become thickened with age, the sunlight would produce a less marked effect; this is exactly what occurs. Up to the present time the amount of alkaloids has annually increased in the bark. In the *C. succirubra* this increase has been determined as carefully as the conditions permit, and the increments during the sixth and seventh years have been 0·75 and 0·5 per cent. respectively. That some part of this increase is due to the inability of sunlight to affect the lower-seated tissues, appears certain from the fact that mossing or otherwise shielding the bark has a less effect in increasing the amount of alkaloids in the older bark than in the thin bark of younger trees. There is every reason to think that the alkaloids will increase by annually diminishing amounts until the amount formed is only equivalent to the destructive influences at work. The growth of the liber and the formation of woody cells, for the reasons given above, is adverse to the increase of yield of alkaloids.

In connexion with this subject it should be mentioned that trees of *C. officinalis*, growing in dry sunny spots, almost invariably produce bark whose main alkaloid constituent is cinchonidine. The trees grown in shade produce more quinine. The bark of trees of rapid and vigorous growth of the same species invariably contain a large amount of alkaloid, and an unusually large proportion of quinine. The latter case can be readily explained by the foregoing principles. The bark of such would be thicker, would contain much parenchymatous tissue, and would, by its more rapid formation, have been exposed for a less time to the deteriorating influences of the sun. It is very remarkable that trees of vigorous growth should yield not only more bark, but also bark in which one hundred parts should contain double the amount of alkaloids.

All cinchona-barks contain small amounts of ammonia. As it is not improbable that ammonia is a stage in the formation of alkaloid, a set of experiments has been commenced as to the action of ammoniacal manure on the yield of alkaloids in the bark; they are, however, not sufficiently advanced to be here detailed.

The Alkaloids considered as substitutes for Mineral Bases.

A speculative opinion has long been held concerning the functions of the vegeto-alkaloids in the plants producing them, whose importance is so great that its practical verification or contradiction is a primary necessity to a scientific theory of the formation of the alkaloids. The hypothesis alluded to is a corollary to the "mineral theory" of LIEBIG, and has been indirectly enunciated by that chemist himself (Chemistry in its Applications to Agriculture, p. 187).

According to this hypothesis, the alkaloids are substitutes for certain of the mineral bases which are the constituents of all plants, and which constitute the larger portion of

the ash obtained when the plants are burnt. The alkaloids possess most of the general properties of these mineral bases; they completely neutralize acids, and as they comport themselves in a manner chemically so similar, it is thought that their functions are identical, and that certain plants are enabled to produce in their own economy actual substitutes for these necessary mineral bases, which are frequently but meagrely furnished by the soil in which the plants grow.

The experiments of PUTTFARCKEN (Pharm. Journ. vol. xi. p. 129) appear to corroborate this hypothesis. They were performed with the dry *Calisaya*-bark from South America, and appeared to show in the main that the bark yielded ash in inverse degree to the amount of organic bases.

The simplicity and beauty of this hypothesis has always rendered it, in the absence of more precise information, a favourite one. On my first acquaintance with living cinchona (*par excellence* the alkaloid-producing plant), there were several circumstances which seemed to show its truth; among these were the exceptionally large yield of alkaloid in the Neilgherry cinchonas, combined with the singular poverty of the soil in lime. These circumstances led me to institute a series of experiments, in which the amount of organic bases was compared with the amount of ash yielded by the same bark; this has been carried on with all the species of cinchona, and all the diversity of conditions I could devise. It is assumed as practically true that the amount of ash is proportional to the amount of mineral bases; and it has been ascertained by experiment that the amount of ammonia is nearly constant, and is too small to vitiate the results. The following Table expresses the results obtained:—

		Alkaloids, per cent.	Ash, per cent.
C. SUCCIRUBRA.			
C. succirubra	June 1867	5·05	2·51
" from Coorg	July "	5·03	2·93
" growing at 7770 feet	August "	2·38	7·80
" " 7660 "	" "	4·21	3·35
" " 5450 "	September "	4·00	2·00
" " 2300 "	November "	4·10	2·43
" " 6900 "	March 1868	5·86	3·17
Mossed bark	" "	10·72	2·25
Bark renewed under moss..........................	" "	8·22	2·20
" " "	May 1869	9·27	2·52
Trunk-bark, 2 years old	March 1868	6·40	3·60
" " 3 "	" "	6·09	3·15
" " 4 "	" "	7·40	2·63
Mean " 7½ "	" 1870	7·55	2·81
" " 6½ "	" "	6·23	2·93
" " 5½ "	" "	7·33	2·97
" " 3½ "	" "	7·61	3·09
" " 2½ "	" "	2·21	4·43
Mean branch-bark, 2½ years old	April 1868	1·91	4·71
" " " 3½ "	" "	4·31	3·13
" " " 7½ "	" 1870	3·20	4·92
" " " 6½ "	" "	2·63	5·00
" " " 5½ "	" "	3·07	3·64
" " " 3½ "	" "	4·96	3·42
" " " 3½ "	" "	0·81	5·03

TABLE (continued).

		Alkaloids, per cent.	Ash, per cent.
Liber of mossed red bark	March 1868	9·80	3·03
External bark of ditto	„ „	11·83	1·80
Liber of red bark	„ „	5·94	2·60
External bark of ditto	„ „	7·89	2·01
Liber 5½ years old	April „	7·10	3·10
External bark of ditto	„ „	8·00	2·38
Bark renewed naturally	March „	5·30	3·27
Bark shielded with tin plate	May „	7·60	2·38
Mean specimen of red bark	„ „	7·43	2·90
Bark from Darjeeling	„ „	6·85	2·70
Prize red bark	September „	8·10	2·10
Mossed ditto	„ „	5·40	1·92
Bark from east of Neilgherries	„ „	6·89	2·17
OTHER BARKS.			
C. officinalis	May 1867	3·72	8·77
„	„ „	4·28	4·49
„	July „	3·84	4·21
„	September „	3·10	3·06
„ at 5500 ft.	„ „	2·68	2·90
„	March 1868	3·22	2·52
„	„ „	2·64	2·97
„ grown in shade	September 1869	3·40	3·89
„	„ „	6·96	3·17
„	„ „	3·77	4·17
„	„ „	7·51	3·00
C. Pahudiana	May 1867	0·13	4·34
„ *nitida*	„ „	5·90	2·14
„ unknown name	„ „	3·96	4·58
„ *peruviana*	„ „	6·16	2·77
Leaves of *C. succirubra*	....	0·019	5·74
Wood „ „	....	0·10	0·89
Bark of willow-trunk	....	0	4·47
„ „ branch	....	0	5·37
„ *Cupressus lusitanica*, trunk	....	0	2·78
„ „ „ branch	....	0	7·72
„ *Rhodomyrtus*, trunk	....	0	3·99
„ „ branches	....	0	5·19
var. "*lanceolata*," trunk-bark	....	5·60	4·54
„ „ „ „	....	11·40	3·34
„ „ „ „	....	11·70	2·62

The hypothesis mentioned above is not borne out by the numbers in the preceding Table.

The nature of the soil on which the trees were grown is throughout the whole very similar. As the great disturbance which might be supposed to occur from variations in the composition of the soil does not take place to any considerable extent under the conditions of the experiment, it is difficult to perceive any cause that can obscure the consequences of the hypothesis, supposing it to be true. But in order to test it still further, the bases in the ash were separately determined in a few cases in which the divergence was most marked, and the numbers obtained were compared with the amount of alka-

loid, but without at all diminishing the discrepancy. I am therefore compelled to believe either that the hypothesis is not true, or else that it is only true to a very partial extent; in fact so partially as to render it of no assistance in the scientific culture of cinchona.

In order to test the hypothesis in another manner, the soil round some young trees of *C. succirubra*, which was naturally singularly poor in lime, was made, by constant dressing with well-exposed hydrate of lime, almost into a calcareous soil. After two years and a half the bark was analyzed and its ash determined. It yielded 6·23 per cent. of total alkaloids and 2·91 per cent. of ash; in other words, the presence of abundance of a powerful base in the soil had affected neither the amounts of alkaloids nor of ash.

The crown barks offer a still further contradiction to the hypothesis. Trees growing close together may have nearly the same amount of ash and yet contain organic bases in nearly the proportion of 1 : 2.

The results of PUTTFARCKEN can be reconciled with mine. His analyses were performed with barks imported in the dry state from South America; among these would be both trunk- and branch-bark. The increased amounts of ash and small amounts of alkaloid in the latter would at first present the appearance of substitution, and would mislead the investigator. I found the same, and was for some time misled also; but a few experiments with the trunk- and branch-barks of various other trees showed me that the latter contain more mineral bases than the former, just as in cinchona, although no alkaloids are present. At the latter end of the foregoing Table some instances of this are adduced.

Furthermore, the fact that the greater part of the alkaloids exists in an insoluble state of combination in the cells of the bark, is itself somewhat adverse to the likelihood of the hypothesis being true; since it is hardly likely that in this situation they can be very active constituents of the plant.