

THE QUANTITATIVE SEPARATION AND ESTIMATION OF THE ALKALOIDS OF COCOA.

BY WILLIAM E. KUNZE.*

THE term "alkaloids," according to the most recent views on the constitution of these bodies, should be reserved for those substances of a basic nature which are found ready-formed in plants, and which are constitutionally derivatives of pyridine. Since theobromine and caffeine are xanthine derivatives, they entirely differ in chemical constitution from the alkaloids proper. They should, consequently, be separated from these and included with the nucleins, which form a subdivision of the proteids. It is a matter of some difficulty to find a generic name for these two bodies which, whilst being precise, clear, and not too cumbersome (cocoa-xanthines?), shall sufficiently define their isolated position and sharply distinguish them from the alkaloids. For this reason the author has retained their old designation (alkaloids) in the present paper. The variety of the methods which have been proposed from time to time for the estimation of these bodies, their number, and the fact that every fresh investigator has propounded a new process, affords distinct evidence that there has not existed, up to the present time, a thoroughly reliable method for their determination. The following is a short *resumé* of the methods which have been hitherto proposed:

1. *Weigmann's Method*.—20 grammes of cocoa are rubbed up with hot water, boiled from a quarter to half an hour, made up to 1 litre, and allowed to settle. 500 c.c. of the clear liquid are precipitated, boiling, with ferric acetate, filtered, the filtrate concentrated by evaporation, 6 per cent. of its weight of H_2SO_4 added and precipitated with phosphotungstic acid. The precipitate is washed with dilute H_2SO_4 , and the amount of nitrogen which it contains estimated; from this the quantity of theobromine deduced.

2. *Mulder's Method*.—10 grammes of cocoa are rubbed up with water, boiled for a quarter of an hour with magnesia, and evaporated to dryness, the dried mass exhausted with chloroform, and the latter distilled off. The residue from the chloroform is dissolved in hot water, filtered, evaporated to dryness, the residue remaining weighed, ignited, and again weighed. The difference between the two weighings gives the quantity of theobromine. The chloroform extract also contains the theine. For its separation from the theobromine Weigmann suggests the following modification: the mixture of cocoa and magnesia is first extracted with benzol (in which theobromine is almost insoluble), the solution evaporated to dryness, the residue shaken out with hot water, the filtrate evaporated to dryness, and the residue weighed as theine.

(Schmidt-Pressler has also pointed out the difference in solubility of the two

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alkaloids in benzol, and called attention to the possibility of their separation in this way.)

3. *Wolfram's Method*.—10 grammes of cocoa, or 20 to 30 of chocolate, are treated for a considerable time with boiling water, ammoniacal lead acetate is then added in slight excess, the mixture filtered hot, and the precipitate washed with hot water until the acidified filtrate, after cooling, no longer gives a precipitate with sodium phosphotungstate.* To the filtrate, which, when excess of lead acetate has been employed, is perfectly brilliant, a solution of caustic soda is added, the whole evaporated to 50 c.c., strongly acidified with H_2SO_4 , and filtered. The theobromine is separated out of the filtrate by the addition of sodium phosphotungstate in considerable excess, with stirring and gentle warming, allowed to become cold, the precipitate filtered off, and washed with a 6 to 8 per cent. H_2SO_4 solution. The washed residue is decomposed by warm barium hydrate solution, the excess of baryta removed with H_2SO_4 , the filtrate neutralized with barium carbonate, filtered hot, and the residue washed with hot water. The united filtrate and washings are evaporated in a platinum dish, dried and weighed. The residue is ignited, the ash treated with ammonium carbonate, the liquid evaporated, and the residue dried, and weighed. The difference between the two weighings gives the quantity of theobromine.

4. *Legler's Method*.—This is essentially a modification of Wolfram's. 20 to 25 grammes of cocoa, or 50 grammes of chocolate, are, after removal of the fat, digested on the water-bath for some hours with 50 c.c. of 4 to 5 per cent. H_2SO_4 , the solution filtered, and the theobromine precipitated by sodium phosphotungstate in excess. The precipitate, which is contaminated with proteids, is allowed to settle for twenty-four hours and filtered off, washed with 8 to 9½ per cent. H_2SO_4 , and dissolved in sodium carbonate solution. The slightly alkaline solution is evaporated to dryness, quartz sand being added, and the residue extracted with amyl alcohol on the water-bath at a temperature of 80° to 90° C.; the solution evaporated in a platinum dish, and the residue dried and weighed. It is then gently ignited and again weighed, the difference giving the theobromine.

5. *Trojanowski's Method*.—A weighed quantity of cocoa in powder is extracted with petroleum spirit to remove fat, well rubbed up with powdered glass and water, mixed with a weight of magnesia equal to that of the cocoa, and dried at a temperature of 60° to 70° C. The dry mass is sprinkled with alcohol and rubbed to a fine powder, boiled with 50 c.c. 80 per cent. alcohol under a reflux condenser for half an hour, this operation being again repeated with another 30 c.c. alcohol for a quarter of an hour. The extracts are united and filtered boiling hot into a beaker, and evaporated on the water-bath; the residue is brought on to a filter with petroleum spirit and washed with alcohol, until the washings run off nearly colourless. The residue on the filter is then weighed, and $\frac{1}{1480}$ of the weight of the alcohol used in washing added as a correction of the solubility of the alkaloid in this fluid.

6. *Zipperer's Method*.—The beans, from which the fat has been previously removed by petroleum spirit, are extracted three times with 80 per cent. alcohol, the extracts united, calcium hydrate added, and the whole evaporated to dryness on

* The sodium phosphotungstate is prepared by dissolving 100 grammes of sodium tungstate and 60 to 80 grammes of sodium phosphate in 500 c.c. of water acidified with nitric acid.

the water-bath. The dry residue is extracted with boiling chloroform in a Soxhlet apparatus. The chloroform extract is evaporated to dryness, and the residue dissolved in boiling water, filtered, and evaporated to dryness in a platinum dish, dried and weighed; 0.89 per cent., representing water, is deducted from the weight found.

7. *Süss's Method*.—6 grammes of an intimate mixture of equal parts of cocoa and quartz sand are extracted for ten hours with petroleum spirit, boiled for half an hour with 200 c.c. distilled water and 6 grammes of freshly prepared pure lead oxide, strained, the residue squeezed, and the fluid thus obtained filtered. The cocoa residue is again twice boiled, each time with 100 c.c. distilled water for a quarter of an hour, and similarly treated. The nearly colourless filtrate is evaporated down to 10 c.c. and shaken out three times with chloroform, 100 c.c. being used each time (time of shaking, three minutes). The bulk of the chloroform removed by distillation, and the remainder evaporated to dryness in a weighed glass vessel.

8. *Diesing's Method*.—The chocolate, after previous removal of the fat by petroleum spirit, is extracted with chloroform for four hours in a Soxhlet, the residue from the evaporation of the chloroform boiled with water several times, and to these solutions the water is added in which the fat had been boiled. The united fluids are then evaporated to dryness in a platinum dish and the residue weighed as pure theobromine.

9. *Bell's Method*.—20 grammes of cocoa are repeatedly digested with benzol, filtered, and the united filtrates distilled; the fatty residue boiled with water, allowed to cool, and filtered. This process is repeated twice, so that the alkaloid may be completely extracted by the benzol. The aqueous extract is then evaporated to dryness, and the residue purified first by solution in water, then in benzol. In this way an alkaloid resembling theine is obtained in white silky crystals. The cocoa, after the treatment with benzol, is rubbed up to a magma with water, sand, and 10 grammes of calcined magnesia added, and the mixture, after being dried in the water-bath, repeatedly treated with alcohol. After removing the alcohol by distillation, the residue containing the theobromine is brought into a glass, dried, and weighed; the last traces of fat and of the other alkaloid removed by treatment with hot benzol, the latter being separated (?) and weighed. The residue insoluble in benzol is twice treated with a little ice-cold water, and in this way the theobromine obtained by decantation is a perfectly pure and white powder, containing a trace of inorganic matter. The amount of the latter is determined by incineration. As even cold water in some cases (?) dissolves a portion of the theobromine, it is advisable to evaporate the aqueous extract to dryness, and if a small quantity of crystals are found, to treat it with water once more (?). "If the whole residue, obtained by extracting the cocoa and magnesia mixture with alcohol, be submitted to a nitrogen estimation, and the nitrogen found calculated to theobromine, a greater and probably more exact percentage amount of the alkaloid would be obtained."

Criticism of the above Methods.—In Legler's method (No. 4) no directions are given as to the nature of the solvent to be used for the extraction of the fat, and this is by no means a matter of indifference, since the alkaloids are soluble in some of these; and, moreover, as Diesing has shown, the solubility of cocoa-fat in the

different solvents varies. Diesing distinctly prefers petroleum spirit to ether or chloroform. Amylic alcohol is an extremely unpleasant liquid to use on account of its physiological effects.

Trojanowski's method (No. 5) is faulty, owing to the necessity of having to make a correction for the solubility of theobromine in the wash-liquid. Moreover, Trojanowski has not used a correct solution-factor, the solubility of theobromine, according to other observers, being only about a tenth of that which he gives, namely, 1 : 12,500 or 16,600. From the author's experiments it is 1 : 7,100 (absolute alcohol at 20° C.).

Zipperer's method (No. 6) is not clearly described; there is no exact statement of the quantities to be used; and there are also other obscurities, to which must be ascribed the low results which he obtained in comparison with those of the author.

Süss evidently came to the conclusion by comparative experiments that the methods of Zipperer and Diesing gave results below the truth. He perceived that the method they employed (direct extraction of the alkaloid with chloroform) was the cause of this; he therefore altered the method of procedure to that of shaking out the alkaloid from its aqueous solution. In this way he obtained nearly twice as much alkaloid as Zipperer and one and a half times as much as Diesing.

After Bell (No. 9) had demonstrated the presence of another alkaloid in cocoa besides theobromine, and Trojanowski had also called attention to the presence of some other body, of which, from lack of sufficient material, he was unable to ascertain the real nature, and after Schmidt had fully identified it with caffeine, it became apparent that the majority of workers had entirely overlooked its presence, and contented themselves with the estimation of the theobromine alone (Mulder, Wolfram). Zipperer expressly insists on the removal by petroleum spirit of the caffeine along with the fat, and his results, therefore, represent pure theobromine, that is, providing the whole of the caffeine is removed in this way. He, however, like the others, entirely neglects to estimate the caffeine. Diesing has remarked that in the extraction of the cocoa-fat a part of the caffeine is also removed; he therefore directs the fat to be boiled in water, and this solution added to that containing the bulk of the alkaloid. He overlooks the fact that it is caffeine which goes over with the fat, and erroneously calls his residue pure theobromine. Since both caffeine and theobromine are precipitated by sodium phosphotungstate, and as they contain different amounts of nitrogen, Weigmann's method must lead to incorrect results. In the methods of Legler, Süss, and Trojanowski the caffeine passes either partially or completely into the fat solution, and escapes detection. The objection to Mulder's method, as stated above, is that the alkaloid obtained is regarded as pure theobromine. This must be taken with a limitation, since, according to the length of time of the extraction with petroleum spirit, the caffeine may be wholly or only partially extracted. According to this, the correctness of the methods of Süss, Trojanowski, Zipperer, and Legler hinges on the fact as to whether the caffeine is partially or totally removed by the petroleum spirit. In the methods of Mulder, Wolfram, and Diesing the results are not reckoned upon pure theobromine, but on a mixture of the two alkaloids. Weigmann's method must be totally rejected. The method of Bell, and Weigmann's modification of Mulder's, possess points of importance.

The author gives the following results, which he has obtained from the same sample of cocoa examined by the different methods :

Method.	Grammes Found.				Expressed in per cents.			Remarks.
	Grammes of Cocoa taken.	Total Alka-loids.	Theo-bromine.	Caf-feine.	Total Alka-loids.	Theo-bromine.	Caf-feine.	
1. Weigmann ...	10	—	0·0652	—	—	0·652	—	N found = 0·0203 grammes, reckoned as theobromine.
2. Mulder mod. by Weigmann	10	0·059	0·0475	0·0115	0·59	0·475	0·115	Estimated by difference.
3. Wolfram ...	10	—	0·215	—	—	2·15	—	Ditto.
4. Legler ...	20	—	0·0785	—	—	0·3925	—	Ditto.
5. Trojanowski ...	10	—	0·564	—	—	5·64 (!!)	—	Coloured deeply brown, greasy, not decolorizable by washing with alcohol.
6. Diesing ...	10	—	0·124	—	—	1·24	—	Coloured, carbonized on heating.
7. Zipperer ...	10	—	0·0235	—	—	0·235	—	Not absolutely colourless.
8. Süß ...	3	—	0·054	—	—	1·80	—	Slightly coloured. A little ash.

The differences in these results are striking, varying, as they do, from 0·235 per cent. (Zipperer) to 5·64 per cent. (Trojanowski). In the determinations by the methods of Zipperer and Legler the theobromine has evidently escaped the solvent action of the alcohol. The author's results obtained by the methods of Zipperer, Diesing, and Süß agree closely with those obtained by Süß, who obtained from 3 grammes of cocoa the following amounts :

Zipperer	0·7 to 0·8%	Theobromine.
Diesing	1·0 „ 1·1	„
Süß	1·4 „ 1·46	„

The analysis by Trojanowski's method gives most improbable results.

The author considers that in any rational method of analysis of the cocoa alkaloids the total quantity of the two together should be first ascertained, one of the bases should then be isolated from this. If, during the fat extraction with petroleum spirit, a partial solution of the theobromine is not to be feared, there yet remains the possibility of small traces being carried over mechanically. The principal reason, which demands that the total amount of the two alkaloids should be first ascertained, lies in the difficulty of removing the caffeine without losing some traces of the theobromine ; and, besides this, there is no criterion to judge when the whole of the caffeine is removed during the fat extraction. The common fault of all the

methods hitherto proposed is that they entirely neglect the estimation of the caffeine, which is a body of no less importance in its physiological effects than theobromine. It has become the rule to estimate the value of such food substances as tea, coffee, cocoa, etc., by the amount of alkaloids which they contain; possibly also the relative proportions of the two alkaloids in a cocoa might give some clue to its origin.

With reference to the influence of cocoa-fat on the estimation, the author has proved that petroleum spirit, either pure or holding cocoa-fat in solution, dissolves caffeine in considerable quantity; Süss has shown that theobromine is absolutely insoluble in these fluids, but he found that small quantities of this body were carried over mechanically in the fat extraction. The author found that the cocoa alkaloids could be readily extracted by a solution of the caustic alkalies with formation of the respective salts, and that the bases were reprecipitated on the alkali being converted into carbonate. He thought that in this way there might be founded a satisfactory process for the estimation of the alkaloids, but the concomitant saponification of the fat proved an insurmountable obstacle. Evidently for a process of this nature to succeed, the fat must be first removed; for this purpose various solvents were tried: benzol, toluol, xylol, chloroform, methyl alcohol, benzyl alcohol, etc., none of which proved suitable, since they all dissolved more or less caffeine or theobromine. The attempt to devise any process which required the previous extraction of the fat was abandoned.

Though the two methods—extraction and precipitation—are equally correct in principle, the latter method is to be preferred in practice, because no solvent is known which will dissolve the alkaloids without simultaneously taking up other substances, and also because with the small quantities of material usually employed the necessary purification of the alkaloid, either by re-crystallization or taking up in another solvent (shaking out), is certain to lead to loss of material unless the work is carried out with the greatest possible care.

The method of determination may be either: 1. Direct weighing; 2. By difference (loss on ignition); 3. Weighing a definite (metallic) compound; 4. By titration.

No. 1 presupposes the purity of the residue; by No. 2 any non-volatile constituent can be deducted; the platinum compounds alone fulfil the conditions of constancy required by No. 3, and permit of a ready control by igniting, and weighing the residue of platinum. The general method of titration proposed by Barth, which depends on the dissimilar reactions of the alkaloids with litmus and phenolphthalein, is inapplicable in this case, since theobromine does not behave towards these indicators in the same way as the other alkaloids. The greater number of the proposed methods prescribe direct weighing; two determine by loss on ignition, one by a nitrogen estimation. Direct weighing possesses the advantage that one has the actual body in hand, which can, when necessary, be identified and tested for purity; but the before-mentioned difficulties in the way of obtaining the alkaloid free from foreign admixtures, and the loss likely to ensue during its purification, considerably detract from its value.

In testing a method for correctness, it is no satisfactory evidence to show that it yields higher results than previous methods; it must rather be demonstrated that the

method is absolutely quantitative and includes no sources of loss or error. This can only be proved by test experiments. In the present case, on precipitating a known quantity of the alkaloid with phosphotungstic acid, regeneration of the alkaloid, etc., the quantity found must exactly correspond with the amount taken. When it has been found that this is so, an investigation must be made to discover if the other bodies present exercise a disturbing influence on the process. In order to determine this, a second control experiment must be made, which shall represent as completely as possible the actual conditions of working. With this object in view the author has made parallel determinations in which a known mixture of the two alkaloids was employed. The difference in the two determinations must, if the process is to be credited with success, exactly represent the quantity of the added material.

From the above considerations a rational process for the estimation of the cocoa alkaloids ought to be founded on the following principles:

I. The estimation, which may be divided into two phases:

1. Determination of the total alkaloids collectively.

2. Their quantitative separation.

II. Previous extraction of the fat is not permissible.

III. The total amount of the alkaloids must be estimated by weight.

IV. The method of estimation must be controlled for exactitude in its various stages and in its whole course by parallel experiments.

V. The alkaloids must be separated in such a form as will permit of their subsequent identification.

In the author's method water is employed as the solvent, since alcoholic fluids and chloroform are excluded on account of the subsequent difficulties in purification, and in order that the fatty and alkaloidal solutions may be kept quite separate. Acid and alkaline solutions were inadmissible, the former because the conversion of the alkaloids into their respective salts might hinder the transference to another solvent, the latter on account of saponification difficulties. The author intended, first of all, to bring the alkaloids into solution with boiling water, then to evaporate the solution to dryness with the addition of a binding material to retain the disturbing admixtures (colouring matters, sugar, etc.), and then exhaust the residue with a suitable solvent. Taking into account the conversion of caffeine into caffeidine, which ensues on long boiling with caustic bases (*e.g.*, $\text{Ba}(\text{HO})_2$), and the decomposition of theobromine on being evaporated with CaO (Diesing), the author made choice of MgO on account of its lesser basicity. Süss has controverted this statement of Diesing's, and to all appearance correctly, for the author found the destruction of the theobromine only partial; he points out that in an estimation conducted according to Zipperer's method the small quantity of theobromine obtained from the residue which contained calcium hydrate points to its partial decomposition, or to the formation of an insoluble calcium compound. He considers that chloroform is to be preferred to amyl alcohol as the solvent. An experiment in which a weighed quantity of theobromine was evaporated to dryness with MgO and extracted with chloroform in a Soxhlet, showed considerable loss, probably owing to the formation of a magnesium compound. On account of the solubility of MgO in boiling chloroform, this fluid could not be used in this condition. Hence the author was obliged to abandon this method and attempt

the isolation of the alkaloids by shaking out with chloroform as proposed by Süss. This necessitated the removal of the colouring matters and other impurities which partially dissolve in that fluid. Treatment with PbO, animal charcoal, or basic ferric acetate, did not suffice to completely decolorize the aqueous solution. The author succeeded in effecting this object by the addition of animal charcoal and aluminium acetate, which, upon prolonged boiling, removed every trace of colour; but traces of tannin and considerable quantities of sugar still remained in solution. The filtrate was then evaporated to 10 c.c., and shaken out several times, each time with an abundance of chloroform; on subsequent evaporation of the united extracts, the residue was not, however, obtained in the requisite state of purity, a brown greasy substance obstinately clinging to it. Probably some reaction took place between the aluminium and the theobromine, for in a control experiment the alkaloid could not be recovered quantitatively. As Mitscherlich has stated, theobromine is retained by animal charcoal; this was proved by direct experiment; of the 0.0375 gramme taken only 0.0057 was recovered. In this method of determination it is assumed that the alkaloids are contained in the cocoa in a form soluble in water; should they be combined with tannin or with organic bases or acids, then it is questionable if they can be extracted by water alone. The precipitation methods offer the essential advantage that an acid medium, which has a more powerful solvent action, may be employed for extraction; the presence of an acid also facilitates the subsequent precipitation. The best reagents for the precipitation are the double phosphates, sodium phospho-tungstate, -molybdate, -antimonate. The precipitate thus obtained can be decomposed by an alkali, when it yields either the corresponding salt of the alkali, or the free alkaloid. When this is effected by NaHO a clear solution results; on passing CO₂ through this the alkaloid is liberated, and a portion, beyond that which is soluble in the medium, is precipitated. The alkaloid may be also liberated by adding to the precipitate a magma of BaCO₃. The precipitation was complete, for in the neutralized residue, obtained by evaporating the filtrate, no trace of alkaloid could be detected by the murexide reaction (chlorine water and ammonia). In a control experiment where a known weight of theobromine was dissolved in about 5% H₂SO₄, the solution treated with phosphomolybdic acid, the precipitate dissolved in NaHO solution, this treated with CO₂, and the residue obtained on evaporation boiled with chloroform under a reflux condenser, the alkaloid was recovered as a pure white powder, containing a minimum amount of ash, thus:

Theobromine taken	0.0605 gramme.
Residue	0.0608 „
Ash	0.0003 „
						0.0605 „
Theobromine found	0.0605 „

In a second trial 0.053 gramme theobromine were taken, and 0.054 gramme theobromine + ash recovered. When the decomposition is effected by baryta water similarly satisfactory results are obtained. It is impossible to obtain the alkaloid absolutely ash-free, consequently in very exact determinations a weighed portion must be ignited and the ash deducted, not forgetting its re-carbonation with ammonium carbonate.

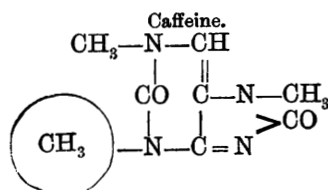
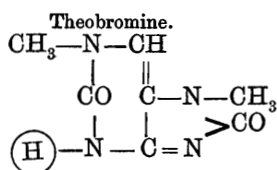
Phosphomolybdic acid was found to be the best precipitant, since the precipitate yielded is of a flocculent nature, and subsides more readily than those obtained by the other reagents. When NaHO is used to liberate the alkaloid, the extraction with chloroform is effected more easily than when Ba(HO)₂ is employed. In the latter case the ash is lower, and is in such minimum quantity that it may be safely neglected. As the decomposition of the precipitate takes place momentarily, the author considers that no action on the caffeine is to be feared. To test the reliability of the process when other substances were present, the author made the following control experiments. The alkaloids from 10 grammes of cocoa were estimated, a parallel experiment being made with the same cocoa to which a known weight of a nearly equal mixture of the two alkaloids was added. The following are the results obtained :

10 grammes of cocoa yielded	0.1195 gramme alkaloids.
Ditto + 0.107 gramme alkaloids	0.2255 " "
Added	0.107 " "
Difference found	0.106 " "

The exact details of the method are as follows: 10 grammes of cocoa are boiled for twenty minutes with about 150 c.c. of 5% H₂SO₄, filtered, and the residue thoroughly washed with boiling water. Excess of phosphomolybdic acid is added, and the whole kept warm for twenty-four hours, filtered off, and washed with approximately 5% H₂SO₄ (800 to 1,000 c.c.). The filter and precipitate are brought, still moist, into a beaker, baryta water added, and CO₂ passed through until the whole of the barium is precipitated. The whole is then evaporated to dryness, dried, the residue carefully removed to a flask, in which it is exhausted with boiling chloroform under a reflux condenser. The chloroform solution is filtered into a small Soxhlet glass, such as is used in fat extractions, the fluid distilled off, and the residue weighed. The mixture of alkaloids thus obtained is invariably pure white, almost ash-free, and gives the murexide reaction. Zipperer found a sticky organic substance which clung persistently to the theobromine obtained by alcohol and chloroform extractions. The author has not found this to occur in quantity sufficient to affect the accuracy of the analysis. The solution of the alkaloidal residue in ammonia is slightly opalescent, foams on boiling, and the theobromine obtained on separation (see further on) was not absolutely white. This may be caused by the body described by Zipperer, or by some other. It does not, however, interfere with the estimation, or with the subsequent separation of the two alkaloids, nor was there observed in the subsequent treatment with ammoniacal AgNO₃ any blackening indicative of reduction, nor was the recovery of the theobromine from the silver salt in any way interfered with by it. The coloured compound of silver and theobromine dissolves in dilute HNO₃ to a nearly colourless solution; by subsequent neutralization, evaporation, and extraction with chloroform pure theobromine is obtained in beautiful crystals.

Having now devised a reliable process for the estimation of the total amount of the two alkaloids, the next point was to find a similarly reliable one for their separation. The simplest would be a physical method, depending on the solubility of one and the insolubility of the other in some fluid such as benzol, petroleum

spirit, etc. Experiments were made with these and many other liquids, but as no satisfactory results could be obtained in this way, the method was abandoned. The attempt to find a reagent which, while precipitating the one, should leave the other intact, failed, it being found that both behaved in a similar manner with the ordinary alkaloidal reagents. Phospho-antimonic, -tungstic, -molybdic acids, and bismuth-potassium iodide (more energetically with theobromine than with caffeine), precipitated both bodies. Iodine and potassium iodide, and gallic acid precipitated neither. The following reagents were also without effect on either alkaloid: alcoholic ammonium sulphide, iodic acid, iodine trichloride, ferrous and ferric chlorides, Millon's reagent, potassium xanthate, basic and neutral lead acetates, chromic acid mixture in the cold, disodium phosphate, potassium ferrocyanide, sodium carbonate, copper sulphate, basic copper acetate. In solutions of theobromine, *but not of caffeine*, mercuric chloride produced a turbidity which formed very slowly; picric acid in HCl solution yielded a gradual crystalline separation; ammoniacal AgNO_3 solution gave, on prolonged boiling, a crystalline precipitate. A solution of *either* alkaloid, when treated with sulpho-molybdic acid, became, on boiling, of a dark-green colour, which spontaneously changed to indigo-blue. Urea and uric acid also gave this last reaction, which may, therefore, be regarded as common to the whole group. The most powerful alkaloidal precipitants, the double phosphoric acids, act alike with the two bodies. The precipitate formed by picric acid with theobromine appeared to consist of a double chloride; on further investigation it did not prove suitable for effective separation on account of its solubility relations. The precipitation with theobromine which takes place with either HgCl_2 or silver solutions is easily explained on comparing the constitution of theobromine with that of caffeine. The former possesses a labile H atom which can be replaced by one of a metal. In the conversion of theobromine into caffeine this is made use of as a stepping-stone, the metal atom being eventually replaced by a CH_3 group.



As these metallic compounds were apparently likely to be suitable for effecting a quantitative separation, the properties of the lead and copper derivatives were also examined, but these were found unsuitable for the purpose, since they were both soluble. The mercury derivative only separated slowly, and in the condition of a fine powder; there remained, therefore, only the silver compound, and this was found completely suitable for the required purpose.

AgNO_3 , when added to an ammoniacal solution of theobromine, gave at first a flocculent whitish precipitate, which redissolved on the addition of ammonia in excess. On prolonged boiling, as soon as the ammonia had been driven off, the solution became turbid, and eventually a crystalline body was precipitated, which the author considered to be the Ag substitution compound of theobromine. It was insoluble in

water or dilute alcohol, easily soluble in ammonia or dilute HNO_3 . It was found to contain no HNO_3 , and must therefore be a substitution compound and not an additive one; 0.2174 gramme of the dry compound yielded 0.1083 gramme AgCl = 0.0815 gramme Ag.

Calculated for $\text{C}_7\text{H}_7\text{AgN}_4\text{O}_2$	37.63% Ag.
Found	37.488% Ag.

Schmidt has described a silver compound of theobromine containing $1\frac{1}{2}$ molecules of water of crystallization; that obtained by the author suffered no loss in weight on prolonged heating at 130°C .

Caffeine, when treated under the above conditions, gave no reaction, and this is readily explained by its not containing a replaceable H atom. The method, therefore, seemed suitable for the separation of the two alkaloids. In order to ascertain the course of the process quantitatively, the author dissolved a known weight of theobromine in ammonia solution, heated to boiling, added AgNO_3 , and continued the boiling until the NH_3 was driven off and the precipitate ceased to increase, filtered off, washed the precipitate with boiling water until the washings were free from AgNO_3 , dried and incinerated filter and precipitate, and weighed the residual silver. Several unsatisfactory experiments showed that in order to ensure success a large excess of AgNO_3 must be used, and that the boiling must be continued until the whole is reduced to a few c.c. Under these conditions good results were obtained, as is shown by the following examples:

1. 0.1195 gramme theobromine yielded 0.0723 gramme Ag, equivalent to 0.1200 gramme theobromine.
2. 0.1570 gramme theobromine yielded 0.0942 gramme Ag, equivalent to 0.1564 gramme theobromine.
3. 0.1432 gramme theobromine yielded 0.0870 gramme Ag, equivalent to 0.1444 gramme theobromine.
4. 0.2394 gramme theobromine yielded 0.1435 gramme Ag, equivalent to 0.2382 gramme theobromine.
5. 0.1165 gramme theobromine yielded 0.0698 gramme Ag, equivalent to 0.1159 gramme theobromine.
6. 0.1240 gramme theobromine yielded 0.0742 gramme Ag, equivalent to 0.1232 gramme theobromine.

The quantity of theobromine equivalent to that of the silver found can be obtained by multiplying by the factor 1.66 ($108 \text{ Ag} = 180 \text{ theobromine}$).

The result of experiments conducted with mixtures of the two alkaloids are equally good:

1. 0.1075 gramme theobromine yielded 0.0645 gramme Ag, equivalent to 0.1071 gramme theobromine.
2. 0.0862 gramme theobromine yielded 0.0517 gramme Ag, equivalent to 0.0858 gramme theobromine.
3. 0.0911 gramme theobromine yielded 0.0543 gramme Ag, equivalent to 0.0901 gramme theobromine.
4. 0.0792 gramme theobromine yielded 0.0479 gramme Ag, equivalent to 0.0795 gramme theobromine.

Further experiments, in some of which the silver compound was collected and weighed in a tared filter, in others where it was dissolved in dilute HNO_3 and the Ag determined as AgCl , yielded equally satisfactory results. In order to shorten these somewhat tedious processes Volhard's volumetrical method was employed in the following manner. A measured quantity of AgNO_3 solution of known strength was added to the solution of the alkaloids, and, after the precipitation and washing, the residual silver in the filtrate and washings was titrated by ammonium sulphocyanate solution, which had been previously titrated against the AgNO_3 solution.* The actual titration was performed in the cold solution, with the addition of 5 c.c. of a cold saturated solution of ferric ammonium sulphate as indicator, and HNO_3 , which had been boiled until it was colourless. This method gives most satisfactory results; it can be quickly performed, and possesses the advantage that the alkaloids can, when necessary, be recovered and subjected to further examination. For this purpose the silver theobromine is dissolved in dilute nitric acid, the solution neutralized and evaporated to dryness; the caffeine solution, in which the titration took place, is also neutralized and evaporated to dryness. The alkaloids can then be easily extracted from the respective residues by chloroform.

The author considers that he has completely solved the problem of the estimation of the cocoa-alkaloids, and that this result cannot fail to influence considerably the future technical valuation of cocoa and its preparations.

W. J. S.

Estimation of Citrate-soluble Phosphoric Acid in Basic Slag and untreated Mineral Phosphates. P. Wagner. (*Chem. Zeit.*, 1894, xviii., 1153-1154.)—The author insists upon the necessity for a guarantee as to the degree of solubility of the phosphoric acid in basic slag, since, either on account of the low basicity of the slag-phosphate, or by reason of the presence of mineral phosphate as an adulterant, samples vary very much in respect of this solubility. Taking the manurial value of the basic slag found to give the best results in field experiments at 100, other samples of the same content of phosphoric acid, and of the same degree of fineness, gave yields represented by 80, 60, 50, and even 30.

The best method of determining the solubility of the phosphate is by the use of a solution of acid ammonium citrate; and if the results are to be unimpeachable, the treatment with this solution should be preceded by a neutralization of the basicity of the slag by citric acid. For this purpose the basicity is first determined as follows: 5 grammes of the slag are introduced into a 500 c.c. flask, which is then filled up to the mark with a 1 per cent. solution of citric acid and shaken for half an hour in a jigger; 50 c.c. of the filtered liquid are then titrated with $\frac{N}{4}$ soda solution, phenolphthalein being the indicator. For the actual analysis 5 grammes of the sample are placed in a 500 c.c. flask with 200 c.c. of water, and there are successively

* The silver solution employed contained 5 grammes AgNO_3 in 100 c.c.; consequently 1 c.c. = 0.03176 gramme Ag; 14.7 c.c. ammonium sulphocyanate solution ($\frac{1}{10}$ normal) = 5 c.c. of the above silver solution = 0.1588 gramme Ag, and 1 c.c. = 0.0108 gramme Ag.

added (1) the quantity of 5 per cent. citric acid solution previously found to be necessary to neutralize the slag, (2) 200 c.c. of acid ammonium citrate solution (see below), and (3) water to the mark. The mixture is shaken in a jigger for half an hour and filtered; to 50 c.c. of the filtrate 100 c.c. of molybdic solution (see below) are added; the liquid is heated to 80° C., and after cooling the precipitate is filtered and treated as usual.

The ammonium citrate solution is made by dissolving 160 grammes of citric acid and ammonia, equivalent to 28 grammes of nitrogen, in a litre of water. The molybdic solution is prepared by dissolving 125 grammes of molybdic acid in a slight excess of 2½ per cent. ammonia solution, adding 400 grammes of ammonium nitrate, diluting to 1 litre, and pouring the solution into 1 litre of nitric acid (1·19 sp. gr.). After having been allowed to remain at 35° C. for twenty-four hours the solution is filtered.

If it be desired to save the half-hour requisite for determining the basicity of the slag, the analysis may be conducted as described above, with omission of the citric acid. In this case an acid ammonium citrate solution containing 150 grammes of citric acid and ammonia equivalent to 23 grammes of nitrogen must be used. The results are less accurate than when the slag is neutralized. A. G. B.

Milk. J. Lehmann and W. Hempel. (*Arch. Physiol.*, 1894, lvi., 558, through *Chem. Zeit.*)—One of the best distinctions between human milk and cow's milk resides in the nature of the casein. Casein from cow's milk contains 6·6 per cent. of calcium phosphate, whilst that from human milk contains 3·2 per cent. The mean ash content of casein from cow's milk is 7·2 per cent. The ash contains:

	Per cent.						Per cent., Calculated on Anhydrous Casein.
CaO	... 49·8	= 3·20
K ₂ O	... 0·9	= 0·06
MgO	... 2·1	= 0·10
Na ₂ O	... 0·4	= 0·03
P ₂ O ₅	... 45·0	= 2·92
SO ₃	... 1·2	= 0·08

It appears that the whole of the phosphorus in the casein molecule is present in the form of a phosphoric acid, and two series of researches gave respectively 1·18 per cent. and 1·50 per cent. of P₂O₅ in the casein molecule. It further appears that casein is a double compound of calcium casein and calcium phosphate in the proportion represented by the formula Ca₃(PO₄)₂, Ca Casein. The proportion of calcium phosphate was not so high in another series of determinations.

The mean ultimate composition of casein is, per cent.:

Ash.	C.	H.	N.	P.	S.
6·47	50·86	6·72	14·63	0·81	0·72

The ash is nearly all calcium phosphate. The ash-free casein contains, per cent.:

C.	H.	N.	P.	S.
54	7·04	15·06	0·847	0·771

The authors do not doubt but that cow's-milk casein and human-milk casein are two individual substances; for not only do they differ in content of calcium phosphate, as shown above, but also in sulphur content, the casein of human milk containing 1.09 per cent. of this element.

The mean results of the many analyses of cow's milk and human milk made for the purposes of this research are as follows:

					Cow's Milk. Per cent.	Human Milk. Per cent.
Casein	3.0	1.2
Albumin	0.3	0.5
Fat	3.5	3.8
Milk-sugar	4.5	6.0
Ash	0.7	0.2
Water...	88.0	88.5

Care was taken that the women were healthy, and that their glands were fully milked.

The coagulation of cow's milk and woman's milk by acids is characteristically different. Whilst the precipitated casein in the cow's milk collects in flocks and sinks to the bottom of the vessel, that in human milk forms a coagulum which does not settle. This difference is traced to the fact that the proportion of fat to casein in the precipitate from cow's milk is so much lower (1.16 : 1) than that in the precipitate from human milk (3 : 1). By the addition of fat to cow's milk the behaviour of human milk when acid is added can be imitated. Dogiel's statement that acids precipitate the casein in coarse flocks from salted human milk is not substantiated.

In order to make an artificial human milk which shall resemble the natural secretion as closely as possible, cow's milk should be diluted with water until its casein content is identical with that of human milk, and cream, milk-sugar, and white of egg should be added until the mixture contains the proportions of fat, sugar, and albumin in human milk. The white of egg should be added in somewhat larger proportion if the suckling is to be fed from the first, since human colostrum, like that of the cow, is particularly rich in albumin.

A. G. B.

Determination of Silicon and Aluminium in Iron. L. L. de Koninck. (*Stahl und Eisen*, 1894, xiv., 138, through *Chem. Zeit.*)—The metal is treated with nitric acid or aqua regia, and precipitated by means of ammonia or by ammonium acetate should manganese be present. The precipitate is dried, ignited, placed in a platinum boat and heated in a stream of hydrochloric acid, whereby ferric chloride is volatilized and a residue of alumina and silica, together with any titanous acid or chromic oxide that may be present, is left. The separation of the substances thus freed from the bulk of the iron is then effected by the usual methods.

B. B.
