	12°.		19°.	
Milk.	0 hrs. x 1000.	12 hrs. x 1000.	0 hrs. x 1000.	12 hrs. x 1000,
Control	15.0	160.0	120.0	112,000.0
Lecithin	15.0	160.0	120.0	95,000.0
Lecithin (2)	15.0	100.0	.....	.....
Choline chloride	15.0	140.0	120.0	104,000.0
Choline phosphate	....	.....	120.0	108,000.0
Coli.				
Control	6.0	9.0	20.0	4,000.0
Lecithin	6.0	7.0	20.0	2,400 0
Choline chloride	6.0	10.0	20.0	3,500.0
Choline phosphate	6.0	9.0	20.0	3,200.0
Typhoid.	(15°).			
Control	23.0	290.0	4.8	137.0
Lecithin	23.0	260.0	4.8	113.0
Choline chloride	23.0	270.0	4.8	105.0
Choline phosphate	23.0	270.0	4.8	127.0

As indicated in the tables, there is, in general, a varying retardation in the development of bacteria in the cultures containing lecithins, although this is not marked in some cases. In fact, in looking over the records of individual plates, it was found that in about 10 per cent. of over two hundred plates no change at all was shown, or there was a very slight increase in the development. Nevertheless, we are inclined to attribute slight bactericidal properties to lecithins at these dilutions, although for practical purposes this is negligible.

In general an effect by choline salts was less frequently observed and with them the diminution was less marked.

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FURTHER STUDIES ON THE APPLICATION OF THE VOLHARD METHOD TO THE ESTIMATION OF ALKALOIDS.

BY ELIAS ELVOVE.

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It has been pointed out by the writer[1] that the difficulty experienced by many workers in obtaining suitable indicators in the alkalimetric estimation of alkaloids and the necessity for using a comparatively large number of such indicators, each more or less suitable to one or only a few alkaloids, may be completely overcome if we adopt the very simple modification of the usual procedure and substitute hydrochloric acid as

[1] Bull. 54, Hyg. Lab., U. S. Pub. Health and Mar. Hosp. Serv., Wash.

the solvent for the alkaloid instead of sulphuric acid which is usually employed.[1] That is, instead of dissolving the isolated free alkaloid in a measured excess of sulphuric acid and determining the excess of acid with the aid of the indicator best suitable for that particular alkaloid, the latter is dissolved in an excess of hydrochloric acid and the excess of acid got rid of by simple evaporation on the water bath, thus obtaining a salt of the alkaloid which, as long pointed out by Plugge[2] and others, may in many cases be estimated with the aid of phenolphthalein as indicator and hence indirectly also the amount of alkaloid. Further, that by thus converting the alkaloids into their hydrochlorides it becomes possible to estimate the alkaloids indirectly through a determination of the chlorine by the beautiful and exact Volhard method. In this way, in addition to avoiding the necessity for using a comparatively large number of different indicators, each more or less suitable to one or only a few alkaloids, we are supplied with a means for estimating such alkaloids as do not react very sharply with any of the indicators thus far studied. Thus of the eleven different indicators which Kippenbeger studied,[3] namely, lacmoid, iodeosine, uranin, cochineal, azolitmin, haematoxylin, methyl orange, ethyl orange, phenolphthalein, alkannin, and Congo red, the only ones which he found to lend themselves in any way for use in the titration of quinine were azolitmin and haematoxylin. The applicability, however, of azolitmin to quinine titrations is denied by Rammstedt;[4] while the misleading results obtainable when haematoxylin is used as indicator in titrating cinchona alkaloids may be seen from the results obtained by Hille,[5] who states that under circumstances which

[1] See U. S. Pharmacopoeia (1905), pp. 28, 67, 107, 143, 146–7, 197, 200, 300, 340, 344. Kippenberger, *Z. anal. Chem.*, **39**, 201–229 (1900). Kebler, THIS JOURNAL, **17**, 822–831 (1895).

[2] *Arch. Pharm.*, [3] **25**, 45–59 (1887).

[3] *Loc. cit.*

[4] *Apoth. Zeit.*, **22**, 1117 (1907).

[5] *Arch. Pharm.*, **241**, 106 (1903). Hille expresses himself in this connection as follows: "Da angenommen wird, das 50 g. genau $^2/_3$ der Chloroform-Aether-Flüssigkeit ist, sollen zur Bindung der Alkaloide 1,3 cm. Säure verbraucht sein. Der Endpunkt der Titration ist nun aber bei Anwendung von Haematoxylin als Indicator sehr schwierig zu erkennen. Ich habe viele Dutzend Titrationen mit Haematoxylin als Indicator auszuführen versucht. Es ist niemals mit Sicherheit zu sagen: Jetzt ist der Farbenumschlag eingetreten. Man wird im allgemeinen bei Anwendung von 1/10 Normallösungen innerhalb eines ganzen Kubikzentimeters im Zweifel sein, ob der Farbenumschlag eingetreten ist oder nicht. Nimmt man jedoch den Punkt als richtig an, wo eben die Flüssigkeit anfängt, die Farbe etwas zu verändern, so bekommt man viel zu niedrige Resultate, wie ich mit reinen Alkaloiden nachgewiesen habe. Von dieser ersten Farbenänderung an ändert sich mit jedem Tropfen 1/10 Normal-Kalilauge die Färbung etwas, so dass man immer im Zweifel ist, wann der richtige Punkt eingetreten ist. Titrationen welche ich mit reinen Alkaloiden ausführte, ergaben Resultate von 85–115 per cent. der angewandten Menge."

should require 1.3 cc. of 0.1 *N* acid, one may be in doubt as to the end reaction to the extent of making an error equivalent to a whole cubic centimeter of the 0.1 *N* solution, which would make the error about 77 per cent. It is probably owing to this apparent lack of a suitable indicator in titrating cinchona alkaloids that the U. S. Pharmacopoeia (1905), at present official, recommends a gravimetric process in all assays[1] where these alkaloids are involved, although it is a recognized fact[2] that the alkaloidal residues obtained in the course of assay frequently contain considerable neutral non-alkaloidal matter, and hence, probably on this account, titration with standard acid is recommended in the cases of most of the other pharmacopoeial alkaloidal assays. Finally, it was pointed out[3] that such procedure applied to the alkaloids would also conclusively show the exact relative acidity of the various alkaloids and the apparent uncertainty[4] as to the exact relative acid-combining power of such alkaloids as quinine and strychnine, for example, would be entirely cleared up.

Recently, Poirrier blue has been recommended by Beckurts and Runne[5] as a better indicator than phenolphthalein in the titration of the salts of such alkaloids as cocaine and also that in certain cases phenolphthalein may be used to better advantage as an indicator if the titration be carried out on an alcoholic solution of the alkaloidal salt instead of the aqueous solution, so that the means for estimating the salts of the alkaloids are still further increased and hence also the advantages of converting the alkaloid into a salt for the purpose of indirectly estimating the alkaloid. However, all these advantages, important as they may be, are nevertheless much more limited in practical application than appears to be the case with the application of the Volhard method for this same purpose. For as pointed out by Plugge[3] and as may be seen from the tabulated results given in the subsequent part of this paper, phenolphthalein does not in all cases afford the means for close quantitative estimation, so that in such cases the results thus obtained are of approximate value only and serve chiefly in indicating about how much standard silver nitrate it will be necessary to add in the subsequent estimation by the Volhard method. On the other hand, the procedure involving the Volhard method affords also the additional advantage of permitting the estimation to be carried out even where it is impossible or difficult to obtain a colorless solution of the alkaloidal residue or its salt—an occurrence which is quite frequently met with in alkaloidal assaying—since by converting the estimation of the alkaloid into a simple determination of the chlorine in sodium chloride

[1] U. S. Pharmacopoeia (1905), pp. 156, 174, 462.

[2] See Kebler, THIS JOURNAL, 17, 830–831 (1895).

[3] *Loc. cit.*

[4] See Schimpf, "Manual of Volumetric Analysis," 5th ed., pp. 502 and 498. Also tables of equivalents in U. S. Pharmacopoeia (1905), pp. 555, 567, 568.

[5] *Apoth. Ztg. (Berlin)*, 24, 662–663 (1909).

which is what the procedure here described practically amounts to, we are no longer dependent on the alkaloidal properties of the alkaloid and hence where its presence interferes it usually may be got rid of by adding sufficient alkali to unite with all of the hydrochloric acid in the solution and filtering off the precipitated alkaloid; or the alkaloid, as well as other organic coloring matter may be got rid of by gentle ignition of the residue obtained on evaporating the solution containing the metallic chloride or the measured excess of standard silver solution. In the previous work[1] along this line, it was shown that this procedure is applicable to the alkaloids quinine, quinidine, cinchonine, cinchonidine, and strychnine. Now the results obtained with eight more alkaloids which are frequently met with commercially, namely, the alkaloids cocaine, morphine, codeine, narcotine, atropine, hydrastine, pilocarpine, and brucine, are presented.

General Mode of Procedure.

Solutions of the respective alkaloids in hydrochloric acid of approximately half-normal strength were carefully prepared so as to make the solution of fiftieth molar strength (one-fiftieth of the molecular weight in grams per liter) with respect to the alkaloid. Varying amounts (5 to 50 cc.) of these solutions were transferred into porcelain evaporating dishes and the latter with their contents placed on the water bath, where they were allowed to remain until the liquid had completely evaporated. To each residue there were then added 5 cc. of alcohol (99 per cent.), with which it was thoroughly stirred and mixed, and the liquid again allowed to completely evaporate on the water bath, when this treatment with 5 cc. of alcohol was repeated. In the case of the alkaloids previously studied, the remaining excess of hydrochloric acid was gotten rid of by simply allowing the residue to remain on the water bath three hours after the liquid had completely evaporated. This latter mode of getting rid of the remaining excess of hydrochloric acid while requiring a longer time than the alcohol treatment is nevertheless simpler in some respects since it requires practically no attention. The chief reason, however, for adopting here the alcohol treatment is because it was found that in the case of some of the alkaloids (*e. g.*, cocaine) considerable of the combined hydrochloric acid is lost if the former procedure is applied. After the second evaporation with the alcohol, the residue was taken up with distilled water (usually 10 cc.), phenolphthalein added, and the acidity of the solution titrated with 0.1 *N* sodium hydroxide. Wherever precipitation occurred on addition of the alkali, the precipitated alkaloid was filtered off, washed with small amounts of water until on testing a drop of the filtrate with silver nitrate it was shown to be free from chlorine, and the chlorine in the solution determined by the Volhard method. The latter was carried out as follows: The solution was diluted to about

[1] *Loc. cit.*

70 cc. with distilled water, then acidified by adding 5 cc. of dilute (10 per cent.) nitric acid, and followed by a measured amount of standard silver nitrate solution which was a little (2 cc.) in excess of that theoretically required. The whole was then made up to a definite volume (100 cc.), filtered through a dry filter, and an aliquot portion (50 cc.) of the filtrate taken for the determination of the excess of silver remaining in solution. The filtering off of the precipitate which usually forms on adding the standard alkali is in most cases unnecessary but was here carried out with all the alkaloids in order to make the procedure more nearly uniform in all cases. With the comparatively larger amounts of brucine the color developed in the solution on addition of the nitric acid interferes in the subsequent determination of the excess silver. Hence in those cases an aliquot portion was evaporated to dryness on the water bath, the residue ignited, then taken up with hot dilute nitric acid and the excess of silver in the solution determined by means of standard thiocyanate in the usual way. The results obtained are given in the following tables.

Cocaine.—A 0.02 *M* solution of cocaine in dilute hydrochloric acid of approximately half-normal strength was prepared, containing 1.212 grams cocaine, $C_{17}H_{21}NO_4$, in 200 cc. The results obtained with this solution are given in Table I.

TABLE I.—EFFECT OF VARYING THE AMOUNT OF COCAINE ON THE CONSTANCY OF THE PROPORTION OF HCl IN THE ALKALOIDAL RESIDUE.

0.02 *M* cocaine taken. cc.	0.1 *N* NaOH required. cc.	0.1 *N* $AgNO_3$ required. cc.	Amount of HCl found.[1] Milligrams.	Theory for $C_{17}H_{21}NO_4.HCl$. Milligrams.
5	0.85	0.96	3.40	3.54
10	2.00	2.00	7.09	7.09
20	4.20	4.10	14.53	14.18
30	6.25	5.96	21.13	21.27
40	8.45	7.90	28.00	28.36
50	11.60	10.24	36.30	35.45

Morphine.—A 0.02 *M* solution of morphine in dilute hydrochloric acid of approximately half-normal strength was prepared, containing 1.140 grams morphine, $C_{17}H_{19}NO_3$, in 200 cc. The results obtained with this solution are given in Table II.

TABLE II.—EFFECT OF VARYING THE AMOUNT OF MORPHINE ON THE CONSTANCY OF THE PROPORTION OF HCl IN THE ALKALOIDAL RESIDUE.

0.02 *M* morphine taken. cc.	0.1 *N* NaOH required. cc.	0.1 *N* $AgNO_3$ required. cc.	Amount of HCl found. Milligrams.	Theory for $C_{17}H_{19}NO_3.HCl$. Milligrams.
5	0.70	1.00	3.54	3.54
10	1.40	2.00	7.09	7.09
20	3.82	4.02	14.25	14.18
30	5.80	5.96	21.13	21.27
40	7.80	8.00	28.36	28.36
50	9.80	10.00	35.45	35.45

[1] All calculations were based on the amount of *N*/10 $AgNO_3$ required.

Codeine.—A 0.02 *M* solution of codeine in dilute hydrochloric acid of approximately half-normal strength was prepared, containing 1.196 grams codeine, $C_{18}H_{21}NO_3$, in 200 cc. The results obtained with this solution are given in Table III.

TABLE III.—EFFECT OF VARYING THE AMOUNT OF CODEINE ON THE CONSTANCY OF THE PROPORTION OF HCl IN THE ALKALOIDAL RESIDUE.

0.02 *M* codeine taken. cc.	0.1 *N* NaOH required. cc.	0.1 *N* $AgNO_3$ required. cc.	Amount of HCl found. Milligrams.	Theory for $C_{18}H_{21}NO_3.HCl$. Milligrams.
5	0.70	1.00	3.54	3.54
10	1.35	2.00	7.09	7.09
20	2.85	3.96	14.04	14.18
30	4.40	5.96	21.13	21.27
40	5.90	8.00	28.36	28.36
50	7.40	9.98	35.38	35.45

Narcotine.—A 0.02 *M* solution of narcotine in dilute hydrochloric acid of approximately half-normal strength was prepared, containing 1.652 grams narcotine, $C_{22}H_{23}NO_7$, in 200 cc. The results obtained with this solution are given in Table IV.

TABLE IV.—EFFECT OF VARYING THE AMOUNT OF NARCOTINE ON THE CONSTANCY OF THE PROPORTION OF HCl IN THE ALKALOIDAL RESIDUE.

0.02 *M* narcotine taken. cc.	0.1 *N* NaOH required. cc.	0.1 *N* $AgNO_3$ required. cc.	Amount of HCl found. Milligrams.	Theory for $C_{22}H_{23}NO_7.HCl$. Milligrams.
5	0.95	1.00	3.54	3.54
10	1.95	2.00	7.09	7.09
20	4.10	4.00	14.18	14.18
30	6.10	5.98	21.20	21.27
40	8.20	7.94	28.15	28.36
50	10.35	9.85	34.92	35.45

Atropine.—A 0.02 *M* solution of atropine in dilute hydrochloric acid of approximately half-normal strength was prepared, containing 1.156 grams atropine, $C_{17}H_{23}NO_3$, in 200 cc. The results obtained with this solution are given in Table V.

TABLE V.—EFFECT OF VARYING THE AMOUNT OF ATROPINE ON THE CONSTANCY OF THE PROPORTION OF HCl IN THE ALKALOIDAL RESIDUE.

0.02 *M* atropine taken. cc.	0.1 *N* NaOH required. cc.	0.1 *N* $AgNO_3$ required. cc.	Amount of HCl found. Milligrams.	Theory for $C_{17}H_{23}NO_3.HCl$. Milligrams.
5	0.10	1.02	3.62	3.54
10	0.20	2.00	7.09	7.09
20	0.80	4.00	14.18	14.18
30	1.10	5.96	21.13	21.27
40	3.60	8 02	28.43	28.36
50	5.30	9.85	34.92	35.45

Hydrastine.—A 0.02 *M* solution of hydrastine in dilute hydrochloric acid of approximately half-normal strength was prepared, containing

1.532 grams hydrastine, $C_{21}H_{21}NO_6$, in 200 cc. The results obtained with this solution are given in Table VI.

TABLE VI.—EFFECT OF VARYING THE AMOUNT OF HYDRASTINE ON THE CONSTANCY OF THE PROPORTION OF HCl IN THE ALKALOIDAL RESIDUE.

0.02 *M* hydrastine taken. cc.	0.1 *N* NaOH required. cc.	0.1 *N* $AgNO_3$ required. cc.	Amount of HCl found. Milligrams.	Theory for $C_{21}H_{21}NO_6.HCl$. Milligrams.
5	1.00	0.98	3.47	3.54
10	2.03	2.00	7.09	7.09
20	4.10	3.98	14.11	14.18
30	6.15	5.96	21.13	21.27
40	8.25	8.04	28.50	28.36
50	10.50	9.90	35.10	35.45

Pilocarpine.—A 0.02 *M* solution of pilocarpine in dilute hydrochloric acid of approximately half-normal strength was prepared, containing 0.832 gram pilocarpine, $C_{11}H_{16}N_2O_2$, in 200 cc. The results obtained with this solution are given in Table VII.

TABLE VII.—EFFECT OF VARYING THE AMOUNT OF PILOCARPINE ON THE CONSTANCY OF THE PROPORTION OF HCl IN THE ALKALOIDAL RESIDUE.

0.02 *M* pilocarpine taken. cc.	0.1 *N* NaOH required. cc.	0.1 *N* $AgNO_3$ required. cc.	Amount of HCl found. Milligrams.	Theory for $C_{11}H_{16}N_2O_2.HCl$. Milligrams.
5	0.98	1.00	3.54	3.54
10	1.95	2.00	7.09	7.09
20	3.85	4.00	14.18	14.18
30	5.90	6.02	21.34	21.27
40	7.90	8.10	28.71	28.36
50	9.95	10.15	35.98	35.45

Brucine.—A 0.02 *M* solution of brucine in dilute hydrochloric acid of approximately half-normal strength was prepared, containing 1.576 grams brucine, $C_{23}H_{26}N_2O_4$, in 200 cc. The results obtained with this solution are given in Table VIII.

TABLE VIII.

0.02 *M* brucine taken. cc.	0.1 *N* NaOH required. cc.	0.1 *N* $AgNO_3$ required. cc.	Amount of HCl found. Milligrams.	Theory for $C_{23}H_{26}N_2O_4.HCl$. Milligrams.
5	0.90	1.00	3.54	3.54
10	1.92	2.00	7.09	7.09
20	4.10	4.00	14.18	14.18
30	6.20	6.10	21.62	21.27
40	8.38	8.10	28.71	28.36
50	10.45	10.10	35.80	35.45

The results given in the accompanying tables show that at least fairly close results may always be obtained if we estimate the chlorine of the combined hydrochloric acid in the alkaloidal residue. It also appears probable that the application of the Volhard method to the estimation of alkaloids is limited only by the possible instability of the hydrochloride

of the given alkaloid under the conditions adopted for getting rid of the excess of the uncombined acid. For estimating the actual amount of alkaloid in specimens of these alkaloids as obtained commercially or in the course of assay, we may therefore adopt the following procedure:

About 0.2 gram of the specimen to be examined is dissolved in an excess of dilute hydrochloric acid (about 20 cc. of 4 per cent.), the liquid completely evaporated on the water bath, the residue thoroughly stirred and mixed with 5 cc. of alcohol and the liquid again similarly evaporated. This treatment with 5 cc. of alcohol is repeated and the liquid again evaporated on the water bath. The residue is then taken up with distilled water (about 10 cc.), phenolphthalein added, and the acidity of the liquid titrated with standard alkali. This titration with alkali will in most cases indicate, at least approximately, the amount of hydrochloric acid that remained combined in the alkaloidal residue and hence may serve at least in indicating how much standard silver solution to add in the subsequent estimation of the chlorine by the Volhard method. Any precipitate formed on addition of the alkali is filtered off, washed with small amounts of water until a drop of the filtrate on testing with silver nitrate is shown to be free from chlorine. The filtrate is then diluted with distilled water to about 70 cc., acidified by adding 5 cc. of dilute (10 per cent.) nitric acid, and followed by a measured amount of standard silver nitrate solution which is judged to be a little in excess of that required to precipitate all the chlorine in the solution. The whole is then made up to definite volume (100 cc.) and filtered through a dry filter. To an aliquot portion (50 cc.) of the filtrate, 1 cc. of a 10 per cent. ferric alum solution is added, and the excess silver in the solution titrated with standard thiocyanate solution. Having determined the amount of hydrochloric acid and knowing that in the case of the alkaloids above mentioned 1 molecule of the hydrochloric acid is equivalent to 1 molecule of the alkaloid, we can of course calculate the actual amount of the latter in the specimen under examination. In the case of brucine, or in any other similar case where the color of the solution would interfere in determining the end reaction, an aliquot portion of the liquid may be evaporated to dryness on the water bath, the residue ignited, taken up with hot dilute nitric acid, and the excess silver in the solution titrated with standard thiocyanate in the usual way.

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