SCIENTIFIC SECTION

THE STRYCHNINE-BRUCINE RATIO OF NUX VOMICA AND THE RELATIVE POTENCY OF THESE ALKALOIDS.*

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In 1818 the discovery of Strychnine (35) and its isolation from St. Ignatius beans (*Strychnos Ignatii*) by Joseph Pelletier, a Parisian retail pharmacist, and Joseph Caventou, also a pharmacist of Paris, was announced. This was followed shortly after, in 1819, by the announcement of the isolation of Brucine from "*False Angustura Bark*" by the same investigators. The alkaloid Brucine was named for James Bruce, a Scottish traveler (44). The first definite clue concerning alkaloids came in 1842 when Gerhardt distilled Strychnine with potash and obtained an oily base, which was called "*Quinoleine*," and later changed to "*Quinoline*" (13). Although at present the alkaloids Strychnine and Brucine are obtained almost exclusively from *Strychnos Nux Vomica*, in which they occur most abundantly in the seeds and in varying smaller quantities in the roots, fruit, pulp, wood, leaves and bark (8), they are found present together in some, as well as separately in other species of *Strychnos;* while in other species there is neither Strychnine nor Brucine, but another substance similar in action to these alkaloids.

Strychnos Ignatii contains on the average of 1.5 per cent of Strychnine and 0.5 per cent of Brucine (27); *Strychnos Tiute* (17) contains 1.4 per cent Strychnine and little or no Brucine; the wood of *Strychnos Ligustrina* contains Brucine only and to the extent of 2.2 per cent, while the bark contains 7.3 per cent of the same alkaloid. The seeds of *Strychnos Rhedii* contain Brucine only, and the same is true of *Strychnos Aculeata* of West Africa. *Strychnos Henningsii* contains neither Strychnine nor Brucine, but another alkaloid instead. *Strychnos Toxifera* and *Strychnos Castelnœi* contain the very toxic "*Curare*" or "*Woorari*," used as arrow poison by South American Indians. *Strychnos Cinnamomifolia*, Thwaites, contains 2.57 per cent of total alkaloids, of which 2.231 per cent is Brucine, and 0.342 per cent Strychnine.

THE STRYCHNINE-BRUCINE CONTENT OF NUX VOMICA.

The Strychnine-Brucine content of Nux Vomica, as given in the literature, varies widely. Evers (9) states that the seeds contain from $2^1/_2$ to 3 per cent of total alkaloids, and that the strychnine content is little less than half, the remainder being brucine. Newcomb and co-authors (30) give the total alkaloidal content as varying from 1.5 to 5 per cent. Hatcher and Wilbert (16) give the strychnine content as 1.25 per cent, but do not give the total alkaloidal, nor the brucine content. Henry (17) states that Nux Vomica contains two or three per cent of total alkaloids, and that the strychnine content is little less than half of the total alkaloidal content, the balance being brucine. Pictet and Biddle (35) say that Nux Vomica contains *about* $1^1/_2$ per cent strychnine and *about* the same amount of brucine.

Billings (3) gives the proportion of strychnine in the seeds as 0.5 to 1.5 per cent, and states that the balance is mostly brucine. Rosenthaler (36) says that, according to origin, samples of Nux Vomica will show a variation of from 0.23 to 5.34 per cent of total alkaloids. He states further that the variety from Bombay shows a variation of from 3.3 to 4 per cent of total alkaloids, the Cochia variety from 3 to 3.5 per cent, and the Madras variety from 2.6 to 3.3 per cent. Hare, Caspari and Rusby (15) believe that both relatively and absolutely the

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alkaloids of Nux Vomica vary most widely. They say that in all probability the difficulty encountered in extraction methods causes analysts ordinarily to fail to obtain full results, that at least 3 to 4 per cent must be considered as existing in seeds of fair quality, and of this, strychnine is ordinarily present to the extent of one-third to one-half, the remainder being brucine. They say that the fixed oil is important from a pharmaceutical point of view, since it is troublesome.

Rusby, Bliss and Ballard (37) give the total alkaloidal percentage as varying from 1.5 to 3 per cent, rarely exceeding this amount. They claim that the relative amounts of the two alkaloids present vary greatly, and that strychnine is usually from one-third to one-half of the total. Scott (41) does not give the percentages of strychnine and brucine present, but states that brucine and strychnine are in combination with igasuric acid (strychnic). Youngken (46) gives the alkaloidal content of Nux Vomica as varying from 1.84 to 5.3 per cent.

Lancaster and Davidson (21) claim that the proportion of strychnine to brucine in the seeds varies as widely as 1.2 to 3.1 per cent. Munch (27) gives the average per cent of total alkaloids in *Strychnos Nux Vomica* as 2.5, consisting essentially of equal parts of strychnine and brucine. He asserts, however, that individual shipments often show a wide variation from this ratio.

From the foregoing references to the literature it appears that the total alkaloidal content of Nux Vomica varies most widely, from 2 per cent to as high as 5.3 per cent. In addition to the variability in total alkaloidal content, the strychnine-brucine ratio also varies widely, in some samples only 0.5 per cent strychnine to 2.5 per cent brucine, in others they are reported in about the same amount, and in some reports the strychnine predominates.

Rosenthaler (36) analyzed four hundred samples of Nux Vomica. The seeds were first pulverized and dried at 100° C. The methods used were those of the Swiss and the German Pharmacopœias, which are in accord with the United States Pharmacopœia so far as alkaloidal requirement is concerned, a total alkaloidal strength of 2.5 per cent being specified in each. The average of his first hundred samples was 2.802 per cent of total alkaloids; the second hundred 2.850 per cent; the third, 2.793 per cent; and the fourth, 2.898 per cent. The lowest yield in the first group of one hundred samples was 2.469 per cent; the second, 2.581 per cent; the third, 2.459 per cent; and the fourth, 1.169 per cent. The highest yield in the first group of one hundred samples was 3.119 per cent; the second, 3.281 per cent; the third, 2.991 per cent; and the fourth, 3.263 per cent. His results showed that of the first hundred samples investigated only one sample assayed below 2.5 per cent, thirty showed an average of from 2.5 to 2.75 per cent, fifty-nine from 2.75 to 3.0 per cent, and only three samples assayed above 3.0 per cent. In the second series none assayed below 2.5 per cent, twenty-five were from 2.5 to 2.75 per cent, sixty-two from 2.75 to 3.0 per cent, and only thirteen samples assayed above 3.0 per cent of total alkaloids. In the third series, again one sample assayed below 1.0 per cent, thirty-seven from 2.5 to 2.75 per cent, sixty-two from 2.75 to 3.0 per cent, and none assayed above 3.0 per cent of total alkaloids. The results of the fourth series show one sample below 2.5 per cent, eighteen between 2.5 and 2.75 per cent, sixty-six between 2.75 and 3.0 per cent, and fifteen samples above 3.0 per cent.

Ericson (10), using the process of analysis as described in the British Pharmacopœia (4), found a variation of 13.1 to 27.5 per cent of total alkaloids in twelve samples of Extract of Nux Vomica investigated. The strychnine content varied from 34 to 88.6 per cent, and the brucine content from 14 to 66 per cent. Extract of Nux Vomica is required to contain not less than 15.2 nor more than 16.8 per cent

of total alkaloids. He also reports that of six samples of tincture and the same number of samples of the powdered drug, the strychnine showed a variation of 45 to 56 per cent, and the total alkaloids present showed a much greater variation. Tincture of Nux Vomica should assay not less than 0.237 nor more than 0.263 per cent of total alkaloids.

In eleven samples of powdered Nux Vomica analyzed by Greenish and Bartlett (14) the total alkaloidal content varied from a low percentage of 2.26 to a high percentage of 2.8.

Lancaster and Davidson (21) report a detailed survey made in the Dominion of Canada on commercial pharmaceutical galenicals. They obtained samples of Fluidextract and Tincture of Nux Vomica from retail drug stores, manufacturers, physicians' supply houses and manufacturing agents. A total of one hundred and six preparations were collected and analyzed, consisting of forty-one fluidextracts and sixty-five tinctures. The methods used were those of the British Pharmacopœia, 1914. The alkaloids were determined gravimetrically. Their results show that twenty-nine (forty-five per cent) of the tinctures assayed within the limits of the British Pharmacopœia. Eight contained strychnine varying from 0.110 to 0.118 per cent, three were over strength, and the balance were decidedly weak. The results of the liquid extract analysis indicate that sixty-three per cent were much too low.

Dufilho (6) conducted a series of assays on samples of Tincture of Nux Vomica, and reports a higher content of brucine than strychnine, but he states that in powdered Nux Vomica there exists more strychnine than brucine. He reports fifty-five parts of strychnine to forty-five parts of brucine, and reached the conclusion that the alkaloidal contents of the commercial preparations, as determined by the methods of the French Codex, do not represent the actual contents of the drugs. He believes the official methods do not completely extract the alkaloids, and that months of time and tons of macerating liquid are required for a complete extraction by macer-percolation.

Schaeffer (39) carried out a series of experiments on powdered, Fluidextract, Extract and Tincture of Nux Vomica. Nine samples of the powdered drug collected at random were assayed first in accordance with the Ninth Revision of the United States Pharmacopœia for total alkaloidal content. The brucine present was then destroyed by treatment with nitric acid, and the assay completed in accordance with the Eighth Revision of the United States Pharmacopœia for strychnine content. It was taken for granted that the other alkaloids were present in such infinitesimal percentages that they did not justify consideration. The results show that all but two of the nine samples came up to the requirements of the U. S. P. IX for total alkaloids, three did not meet the specifications of the U. S. P. VIII for strychnine content, while two others met the U. S. P. IX requirements but not those of the VIII. Still another failed to pass either the old or the new requirements, while the last passed the U. S. P. VIII requirements but not the IX. In some cases his results on powdered Nux Vomica show the total alkaloids present as high as 3.53 per cent, and in others as low as 1.92 per cent. The strychnine content showed a variation of 0.73 per cent, the brucine content 1.12 per cent to 2.23 per cent. The assays of the Fluidextract, Extract and Tincture were carried out in the same manner. Results of the fluidextract analysis showed a variation in total

alkaloidal content of from 2.37 to 2.63 per cent. The strychnine content showed a variation of from 0.79 to 1.21 per cent, and the brucine, by difference, from 1.20 to 1.67 per cent. With six samples of the Extract, a variation in total alkaloidal content of from 14.89 to 17.1 per cent was found, and the strychnine varied from 8.89 to 10.48 per cent. Six samples of Tincture of Nux Vomica assayed from 0.237 to 0.263 per cent total alkaloids, the strychnine content from 0.080 to 0.114 per cent, and the brucine, by difference, from 0.110 to 0.171 per cent. In the series of analyses the percentage of strychnine and brucine varied from 33 to 53 per cent. Schaeffer, therefore, concluded that there is absolutely no relationship between the percentage of alkaloids (total) and that of strychnine.

The beginning of a new pharmacopœial decade, and the organization of the work involved in the revision of U. S. P. X has been attended by numerous proposals looking toward improvements in and modifications of present standards, the physical and chemical properties of galenical preparations, and the methods of standardization of pharmacopœial agents. Among the many suggestions which have been made, appears the proposal to return to the old method of standardizing Nux Vomica according to the strychnine-content only. The claim has been made that the present standards for the chemical assay of Nux Vomica are not full criterions of the pharmacodynamic activity of the crude drug and its preparations; neither do they represent their true physiological properties.

THE RELATIVE PHARMACOLOGIC ACTIVITY OF STRYCHNINE AND BRUCINE.

The general opinion concerning the relative pharmacologic action of strychnine and brucine is that the action of strychnine is many times greater than that of brucine. Billings (4) says that the action of brucine is many times more feeble than that of strychnine. Pictet and Biddle (35) state that the actions of strychnine and brucine are the same, but that brucine is many times less active than is strychnine. Hatcher and Wilbert (16) do not give a relative activity-ratio for strychnine and brucine, but state that brucine is of very minor importance since it contributes little to the action of the crude drug, and that strychnine is many times more active than brucine.

Lancaster and Davidson (21) state that strychnine is forty times more potent than brucine. According to Munch (27), brucine is much less potent than strychnine physiologically, and the determination of total alkaloids in Nux Vomica does not insure constancy of physiological action. Jenkins and DuMez (18) say that, from the standpoint of toxicity, brucine is eight times less active than strychnine. The official method of assay, according to them, is therefore not a correct measure of the therapeutic activity of Nux Vomica.

Washburn and Blome (44) give the relative activity of brucine to strychnine in a ratio of one to sixty. Wood and LaWall (45) claim that it is difficult for physiologists to determine the exact seat of action of pure brucine upon the human organism, since it is very difficult to separate it from strychnine, and that it was formerly thought that brucine was much less poisonous and acted as a depressant to the peripheral sensory nerves.

By using four specimens of brucine, the first three unwarranted and the last one warranted by Merck and Company, Mays (24), experimenting on frogs and using the nasal reflex method, showed that unwarranted specimens of brucine con-

tained strychnine as a contaminant as was evidenced by its typical action when larger amounts of the so-called pure brucine were used. He concluded that strychnine and brucine have a few points in common, but possess so many dissimilar ones that there is sufficient grounds for believing that their difference is not one of degree only. He states that the convulsions which appear early in strychnine poisoning, if at all, occur late in brucine poisoning; and that convulsions invariably develop before death in strychnine poisoning, while not infrequently in brucine poisoning death occurs without even a trace of spasm.

Zeiss (47), using a five per cent solution of pure brucine, found that on the unbroken skin it produced a local analgesic-anæsthetic effect, and in only two or three instances did the subjects with whom he worked complain of any degree of nervousness. He is of the opinion that such an effect in these cases was caused from the strychnine-like effect of the drug. He applied the solution to painful furuncles of the external auditory canal, with the result that in all cases the subject manifested almost immediate relief. In some fifty subjects to whom he applied the five per cent solution to painful surfaces, caused from the application of silver nitrate, copper sulphate and iodine, all were afforded relief. He believes that the analgesic effect produced by the five per cent solution of brucine is not equal to that produced by cocaine hydrochloride, that it is more lasting, but is much less reliable, and that the drug is not readily absorbed.

Lyons (22) states that the medical activity of Nux Vomica and the allied drug Ignatia is due chiefly to the strychnine which they contain; that brucine has a share in that activity but is comparatively unimportant, and that the relative proportion in which these alkaloids are present is so nearly uniform in the respective drugs that the percentage of strychnine furnishes a practically correct measure of the therapeutic value of a sample of either drug. He believes that, for comparison, the content of strychnine is a better criterion than that of total alkaloid.

From the data presented, it is obvious that there is much controversy regarding the total alkaloidal content, as well as the relative amounts of the two major alkaloids, strychnine and brucine, in samples of the powdered drug, the Fluidextract and Tincture of Nux Vomica. It seems, also, that the relative pharmacologic actions of these alkaloids, as described in the literature, vary most widely; some writers stating that brucine is eight times less toxic than strychnine, while others present the relative degrees of toxicity as one to sixty.

Fortunately or unfortunately the International Standards for Nux Vomica and its preparations are based on total alkaloids, and these have, therefore, become almost universally official. The British Pharmacopœia (4) adheres to a strychnine standard. In the current revision of the United States Pharmacopœia (10th) the standards of assay for Nux Vomica and its preparations are based upon the total alkaloidal content, the Pharmacopœia having abandoned the strychnine standard,[1] beginning with the Ninth Revision, in order that the standards of assay would conform with those of the International Standards. Nux Vomica and its galenicals are listed in the International Protocol (34), indicating that they are in agreement with the international requirements for alkaloidal strength.

The purposes of this investigation, therefore, were (A) to make a wide collec-

[1] The standard of assay in the Eighth Revision of the U. S. P. was based on Strychnine content alone, the requirement being not less than 1.25 per cent (33).

tion of samples of powdered Nux Vomica, manufacture the official preparations of the drug that are most frequently employed in therapeutics, and carry out proximate assays on the drug and preparations of the drug, first for total alkaloidal content, and then for the relative amounts of strychnine and brucine present, in order that some data might be derived to show the actual ratio existing between strychnine and brucine in Nux Vomica, and (B) to determine the relative pharmacologic activity of strychnine and brucine and their salts by means of various laboratory animals.

MATERIALS.

Samples of the crude powdered drug, Nux Vomica, were secured from eight different market sources. Microscopic examination[1] of each sample established its freedom from adulteration. A fluidextract and a tincture were made from each sample, using the United States Pharmacopœia X and National Formulary V methods (34, 28).

Samples of Strychnine, Brucine, Strychnine Sulphate, Strychnine Nitrate, Brucine Sulphate and Brucine Nitrate were purchased on the market, and their purity and strength established according to the pharmacopœial and National Formulary methods (34, 28). These alkaloidal agents were used for checking the methods of alkaloidal extraction employed in this investigation.

CHEMICAL METHODS (PROXIMATE ASSAYS).

The United States Pharmacopœia first introduced drug assays about fifty years ago, but studies of the methods of extraction of alkaloidal-bearing drugs have been undertaken for more than 125 years (43).

Among the various methods for the extraction of the alkaloids of Nux Vomica which have been proposed from time to time, is the automatic extraction method of Palkin, Murray, etc. (31, 32), devised to replace the tedious hand operations. Complicated construction, lack of effectiveness and limitation of automatic features are the major criticisms of this method. The short period of extraction, one to two hours, is its chief advantage.

McGill and Wagener (26) and McGill and Faulkner (25) applied electrometric titration methods to Nux Vomica and its preparations. McGill and Wagener's method yields values that are higher than those obtained by the official methods, while the method of McGill and Faulkner gives values which compare favorably with those obtained by the official methods. The period of time required for the purification of the alkaloids is shortened by these methods.

Beal and Lewis (2) are of the opinion that the more practical and more accurate methods of assay are those based upon the facts that most alkaloids are insoluble in water but soluble in organic solvents, while most of their salts are soluble in water but insoluble in organic solvents. The official methods are based upon these general principles, and these methods are known as the "shaking-out methods." Such methods assume that the alkaloid is insoluble in neutral aqueous solutions, and that the alkaloidal salt is almost insoluble in the immiscible solvents used. The official methods assume also that the salt is neither hydrolyzed by the aqueous solvent nor decomposed by the organic solvent.

[1] By A. John Schwarz.

The rate of extraction of the alkaloids by means of the immiscible solvents depends upon the coefficient of distribution for alkaloid between the two immiscible liquids, a constant which has been given the name "extraction factor," meaning the ratio of the amount of alkaloid in the layer of added solvent to the amount that was originally present in the first solution. Beal and Hamilton (1) claim that this term is more expressive than "partition ratio," since the latter tells only the partition for one molecular species between two layers of equal volume, while "extraction factor" tells at a glance the completeness of the extraction.

In the extraction of alkaloids from Nux Vomica, advantage is taken of the fact that they occur in the form of salts which are soluble in water or alcohol, or a mixture thereof, and may be removed by a mixture of these two solvents. This is done in the official methods for the preparation of tinctures and fluidextracts.

When the alkaloids, strychnine and brucine, are extracted from the crude drug or their preparations, they are accompanied by many other forms of extractive matter (1), which consist essentially of albumin, proteins, fats, resins, gums, organic acids, coloring matter and carbohydrates. The carbohydrates do not interfere with extractions, but the other elements must be removed or the final estimation, whether determined by gravimetric or volumetric methods, will not be accurate.

Sabalitschka and Jungermann (38) recommend a method of extraction which first removes the fixed oil with petroleum ether, the alkaloids present being determined by volumetric methods. The removal of the fixed oil greatly facilitates the assay process and gives more accurate results, since the presence of a fixed oil causes the results to run high.

Beal and Hamilton (1) found that lead acetate, when used as a clarifying agent for alkaloids, causes a removal of the coloring matter and organic acids, but has no harmful effect on the alkaloids. They report also that the addition of an aqueous solution of sodium chloride, half saturated, causes the removal of a greater amount of alkaloids at a single extraction.

The use of very strong or very weak solutions of ammonia has been suggested as a great source of error in alkaloidal assays because of the formation of tedious emulsions which do not readily separate. Goldberg (12) found that very troublesome emulsions were formed when ammonia water between 6.2 and 8.3 per cent in strength was used. Ammonia waters of 9 per cent and 9.96 per cent strength gave the most satisfactory results. With these two strengths any slight emulsions formed were easily broken up with but little deposition of extractive matter. Ammonia water stronger than 10.5 per cent precipitated a considerable amount of extractive matter and caused very troublesome emulsions. It is his opinion that the official ammonia water, containing between 9.5 and 10.5 per cent of ammonia, gives best results with the least amount of inconvenience.

In the determinations of the alkaloids of powdered Nux Vomica and its preparations, there seems to be a great divergence in opinion regarding the estimation of the alkaloids by volumetric and by gravimetric means. There is likewise a difference in opinion regarding the method that should be employed for the destruction of brucine in a common strychnine-brucine residue. Dufilho (7) favors the volumetric method as the more satisfactory, and recommends that the brucine be destroyed with as many times 15 cc. of a mixture of equal parts of nitric acid and

water as there are grams of alkaloid. The red liquid then is to be rendered alkaline with sodium hydroxide after a period of one hour, and the strychnine determination made by the titration method.

Schimpf (40) claims that, because of the fact that the alkaloidal residue is rarely, if ever, in an absolutely pure condition, gravimetric results are by no means correct and may vary widely. He believes that the volumetric method is more satisfactory.

Fuller's (11) opinion, based on the results of collaborative work, is that greater reliance can be placed in a gravimetric method.

Lachaise (20) criticizes the standardization of the strychnos drugs based on the volumetric determination of total alkaloids on the grounds that the accepted equivalent, 364 (the arithmetical means of 395 and 334, the respective molecular weights), is necessarily inaccurate, since the two alkaloids are rarely, if ever, present in equal proportions. He states further that the chemical standard, based on strychnine content, accords better with the physiological standard, since the physiological action of strychnine is not greatly affected by brucine.

The British Pharmacopœia (4) directs that brucine be destroyed in the strychnine-brucine residue by adding three cc. of a mixture of equal volumes of distilled water and nitric acid at a temperature of 40° C. for a period of ten minutes, the solution then transferred to a separatory funnel and rendered alkaline with sodium hydroxide, and shaken out with 10-, 10- and 5-cc. portions of chloroform, and the strychnine determined by weight after drying to constant weight at 100° C.

There are numerous criticisms throughout the literature of the British Pharmacopœia method for the destruction of brucine in a common strychnine-brucine residue. Dott (5) recommends that brucine be destroyed at ordinary rather than at an elevated temperature, and that twenty minutes is a sufficient length of time to cause the reaction to be complete when 1 cc. of concentrated nitric acid is used for each 0.25 Gm. of brucine.

Short (42) found, in running parallel assays on the destruction of brucine by the B. P. Method, that the lower temperature in nitration gave better results in estimation by weight than by the volumetric method. Jenson (19) also criticizes the method of the B. P., claiming that with known amounts of strychnine and brucine he showed a loss of 9.9 per cent strychnine, mainly caused by nitrations carried out at 50° rather than at 20°. By improving the methods of oxidation (lowering temperature) results were correct to within 1.75 per cent, while in the parallel 50° nitration, the loss in the recovery of strychnine was 11 per cent. He favors the volumetric method for the estimation of strychnine, claiming that gravimetric results are 8.8 per cent too high with known amounts of the alkaloids employed.

Lyons (23) reports that in the assay of Nux Vomica and its preparations the brucine is destroyed by oxidation with nitric acid having a specific gravity of 1.42, and that the reason for non-destruction of all the brucine is due to a lack of nitrous acid in the nitric acid. Some investigators have added a trace of sodium nitrite to start the reaction, but he employed powdered sugar, 10–20 milligrams, and obtained good results.

Nelson (29) gives a method for the destruction of brucine in the combined alkaloidal residue with 18 cc. of a mixture of equal parts of 2.5 per cent sulphuric and 2.5 per cent nitric acids, the acids to stand in contact with the alkaloids for a

period of ten minutes, and the strychnine solution then transferred to a separatory funnel, rendered alkaline, and re-extracted and determined.

Lyons (22) also gives a method for the destruction of brucine by using 1.5 cc. of strong nitric acid and 1 cc. of a 5 per cent solution of sodium nitrite in water, the mixture to be stirred well and allowed to stand for exactly ten minutes to cause a complete destruction of the brucine.

In this investigation, a preliminary study of the methods of Nelson, Lyons, the British Pharmacopœia and the U. S. P. VIII for the destruction of brucine on known amounts of strychnine and brucine was carried out. By Nelson's method only 2 per cent of known amounts of brucine was destroyed. This method apparently does not have sufficient nitrous acid in the nitric acid to cause the reaction to start. The U. S. P. VIII method showed a destruction of only 93 per cent brucine. The British Pharmacopœia method showed a complete destruction of brucine, but also a destruction of five per cent of the strychnine present. Lyons' method, a modification of the British Pharmacopœia method, showed a complete destruction of brucine and a complete recovery of the strychnine present in known samples of the combined alkaloids. Lyons' method, therefore, was the one employed in this investigation for the destruction of brucine in the combined strychnine-brucine residue.

Samples of powdered Nux Vomica, Fluidextract and Tincture of Nux Vomica were assayed first for total alkaloids by the U. S. P. X methods with one modification, *viz.*, the omission of the final volumetric step. The combined alkaloidal residue was treated with one and one-half cc. each of strong nitric acid and five per cent solution of sodium nitrite in water, the mixture stirred well and allowed to stand for exactly ten minutes at room temperature to cause the destruction of the brucine. The strychnine solution was then rendered alkaline with 10 per cent Ammonia Water, and the strychnine re-extracted and weighed, after drying to constant weight at 100° C., and estimated as the free alkaloid.

The methods employed in the assay of the powdered Nux Vomica, Fluidextract, and Tincture of Nux Vomica follow:

A.—POWDERED NUX VOMICA.

The U. S. P. X method (34) for total alkaloids was followed, the only modification being the determination of the alkaloids by gravimetric means only. The chloroformic solutions were filtered into a tared beaker, evaporated to dryness on a water-bath at a temperature not exceeding 60° C., then dried in an oven to constant weight at 100° C., and weighed.

In the extraction of the alkaloids with sulphuric acid, seven portions of the solvent were used, this amount, being necessary to cause complete extraction of the alkaloids. 20-, 20-, 15-, 15-, 10-, 10- and 5-cc. portions of the acid were used.

During the chloroformic extraction of the free alkaloid from the alkaline liquid, usually seven portions were necessary to cause a complete extraction of the alkaloidal bases, and 25-, 20-, 15-, 15-, 10-, 10- and 5-cc. portions were employed.

DETERMINATION OF STRYCHNINE AND BRUCINE IN THE COMBINED RESIDUE.

The combined residue was treated with 3 cc. of a mixture of equal parts of nitric acid, having a specific gravity of 1.42, and a five per cent solution of sodium

nitrite in water. The mixture was kept at room temperature, and the combined solutions allowed to stand in contact with the residue for a period of exactly ten minutes. The red solution of strychnine was then transferred to a separatory funnel, washing all traces of the alkaloid from the vessel, rendered alkaline with Ammonia Water, and completely extracted with chloroform (20, 15, 10 and 5 cc. usually being a sufficient amount to cause complete extraction), filtering each portion as drawn off into a tared beaker. Extraction was carried out until Mayer's Reagent showed that extraction was complete. The chloroform was then evaporated off, and the residue weighed. The brucine was determined by difference.

B.—FLUIDEXTRACT OF NUX VOMICA.

The National Formulary method (28) was employed in the assay of Fluidextract of Nux Vomica, the only modification being the determination of the alkaloids by weight rather than by the volumetric method given.

DETERMINATION OF STRYCHNINE AND BRUCINE IN THE COMBINED RESIDUE.

The method described under powdered Nux Vomica was employed, the only difference being that more chloroform was used to completely shake out the strychnine, 20-, 20-, 15-, 15- and 10-cc. portions having been used. The strychnine was determined again by weight, and the brucine by difference.

C.—TINCTURE OF NUX VOMICA.

The method employed in the assay of Tincture of Nux Vomica was that of the U. S. P. X (34) for total alkaloids, the brucine then destroyed by oxidation with nitric acid and sodium nitrite, and the strychnine shaken out and determined by weight. The only modification of the official method was the final determination of the alkaloidal residue by weight rather than by volume, as the Pharmacopœia specifies.

DETERMINATION OF STRYCHNINE AND BRUCINE IN THE COMBINED RESIDUE.

The method which has been described under powdered Nux Vomica and Fluidextract of Nux Vomica was used also in assaying the Tincture.

PHARMACOLOGIC METHODS—A. FROGS.

The idea presented itself that the possible reason for such a wide difference in opinion regarding the relative toxicity of Strychnine and Brucine and their respective salts was due to the particular specie of laboratory animal with which various investigators have worked, and the specie susceptibility of the particular type of animal employed. Most of the authors consulted do not state the type of animal used in any comparative work. In this investigation the pharmacological studies were carried out on four different species of common laboratory animals, *viz.*, the frog, the rabbit, the dog and the cat.

TECHNIQUE OF THE FROG METHOD.

The common grass frog, *Rana Pipiens*, was employed in these observations. They were obtained from Wisconsin, and stored in the frog ponds until ready for use.

The animals were then removed, the excess water wiped off, and the animals accurately weighed in a tared beaker to the first decimal place.

The solutions of strychnine sulphate and brucine sulphate for hypodermic injection were made up in sterile physiological saline solution, and the concentration so adjusted that each animal would receive approximately the same amount of fluid (about 0.5 cc.).

The fluid containing the alkaloidal salt was injected into the ventral lymph sac by means of a Luer type hypodermic syringe. A preliminary series of observations was conducted in order to arrive at the comparative convulsive dose of the drug. Observations were made continuously after the injection of the dose, and the time was noted when the animal became hypersensitive, and when the first convulsion occurred.

B.—RABBITS.

Rabbits used in the observations were obtained from local sources. The weight of each animal was ascertained in kilograms. The solutions of strychnine and brucine sulphates were made up in sterile, physiological saline solution, as described in the technique of the frog method. The dose was administered by subcutaneous injection in the abdominal region with a sterile hypodermic syringe, the concentration being adjusted so that each animal received approximately the same volume of fluid.

Observations were made constantly, and the time to hypersensitivity, and to the first convulsion were recorded.

C.—DOGS.

The weight of each dog was determined in kilograms, and the solutions of strychnine and brucine sulphate prepared in sterile, physiological saline solution, and injected by means of a sterile syringe subcutaneously in the abdominal region.

Observations were made constantly, and the time recorded when the animal appeared hypersensitive, and when the first convulsion occurred.

D.—CATS.

The same technique was employed with cats as has been described under "rabbits" and "dogs." Observations again were made constantly, and the length of time to hypersensitivity, and the time to the first convulsion were noted.

(To be continued)

A STUDY OF THE PREPARATION, QUALITATIVE DIFFERENTIATION TESTS, AND METHODS OF EVALUATION OF THE VARIETIES OF ALOE.*

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF MINNESOTA.[1]

BY KARL GOLDNER.

Aloe, U. S. P. X (1), is the inspissated juice of the leaves of *Aloe Perryi* Baker, known in commerce as Socotrine Aloe; of *Aloe vera* Linné, known in commerce as

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