for estimating yellow phosphorus in pharmaceutical preparations and especially the process requiring the destruction of the organic matter and oxidation of the phosphorus by the Kjeldahl method. The fact that the results obtained by this method were not at all concordant may be due to a too rapid oxidation of the phosphorus which manifests itself at times by small explosions.

We have found that with the method outlined by us we can get uniform and satisfactory results and when you get results which remain within the 5 per cent limit and taking even the personal equation into consideration within the 8 per cent limit you should be very well satisfied.

A Member: Are these phosphorus compounds, that is, the therapeutic products, pills, etc., used as extensively as they were some years ago? What is the demand?

Dr. Engelhardt: My impression is that we still make rather large quantities of pharmaceutical preparations containing phosphorus, especially those containing in addition to phosphorus, extract of nux vomica and extract of damiana. On account of the instability of phosphorus in elixir of phosphorus, nux vomica and damiana we have quite recently replaced the phosphorus by glycerophosphates. Such a preparation is apparently equally as effective, for we continue to have a great demand for it.

A Member: It is perhaps not germane to the subject, but I should like to ask whether there is any real evidence as to the therapeutic value of either elixir of phosphorus or glycerophosphates?

Dr. Engelhardt: You had better ask the American Medical Association. They claim not.

SEMPERVIRINE FROM GELSEMIUM ROOT*.

A. E. STEVENSON AND L. E. SAYRE.

The authors give a method of separating "Sempervirine" from a mixture of the combined alkaloids of gelsemium root, by means of converting the alkaloid into the nitrate which is quite insoluble in water and practically insoluble in solution containing sodium nitrate. Methods of preparing the free alkaloid and some of its salts, a description of these products, and their reactions with the usual alkaloidal reagents are given. In the second part of the paper the authors describe gelseminine which, they claim, consists of at least two alkaloids. They further suggest a method for separation of the various alkaloids of the root and the results of some preliminary physiological experiments carried out with sempervirine, which have shown that the salt apparently has no *immediate* toxic effect, but its definite toxicity is stated.—(Editor.)

At previous annual meetings of this Association, the progress in the investigation of Gelsemium Root has been contributed. In former papers, the various contributions of different authors have been referred to and, at this time, it is unnecessary to repeat the same. It is the desire of the present authors to record in the proceedings of this Association any results of this investigation.

In the present paper, we desire to report further progress in the separation of the various alkaloids of this interesting drug and will confine ourselves very briefly to the method of separation of the alkaloid sempervirine from the total alkaloids of Gelsemium. This report, taken in connection with former

*Presented to Scientific Section, A. Ph. A., San Francisco.

reports, will reveal consecutive steps which have been taken in this investigation.

A.—The crude mixed alkaloids of Gelsemium weighing 25 grams were dissolved in chloroform and extracted with one percent citric acid until practically all the alkaloid was removed from the extraneous material. The acid liquid was made alkaline with ammonia water and completely extracted with chloroform. The alkaloid was then again extracted from the chloroform using one percent hydrochloric acid. Each successive extraction was tested by adding a few drops of saturated solution of sodium nitrate which precipitates the sempervirine. When no further precipitate was obtained the extracts previously obtained were combined and a saturated solution of sodium nitrate added, drop by drop with stirring, until no further precipitate was obtained. This precipitate consisted of sempervirine nitrate. The alkaloid, as stated in previous papers, forms a nitrate, quite insoluble in water and practically insoluble in solution containing sodium nitrate.

B.—Purification of the Sempervirine Nitrate. The sempervirine nitrate was filtered off and washed free from mother liquor with a weak solution of $NaNO_3$ and the $NaNO_3$ solution removed by washing drop by drop with distilled water. The sempervirine nitrate was washed with distilled water into a beaker and heated on a boiling water bath until it dissolved. The solution was filtered off and the nitrate reprecipitated by adding a few drops of a saturated solution of $NaNO_3$. It was then filtered off and washed as before with weak $NaNO_3$ solution. This process of solution and reprecipitation was repeated three or four times. Finally the sempervirine nitrate was dried on porous plate and recrystallized from hot alcohol from which it separated in yellow needle shaped crystals.

C.—Preparation of the Free Alkaloid. The nitrate was dissolved in hot water, the solution made alkaline with NH_4OH and the alkaloid extracted with $CHCl_3$. The major portion of the $CHCl_3$ was distilled off. On allowing the residue to cool the alkaloid crystallized out in reddish brown needles.

D.—The Hydrochloride, Preparation of. The hydrochloride was prepared from the free alkaloid in a manner analogous to the preparation of the nitrate, the alkaloid being dissolved in one percent HCl and precipitated out by adding saturated solution of NaCl. It was dried on porous plate and separated from the NaCl present by dissolving in alcohol in which it is quite soluble.

It may be obtained in a crystalline form by dissolving in a small amount of absolute alcohol, heating the alcohol solution to boiling and adding 4 or 5 volumes of hot $CHCl_3$. On cooling and standing for some time, the hydrochloride separates out in yellow microscopical needles. The hydrochloride dissolves readily in alcohol and water. It is very slightly soluble in chloroform.

E.—Some Characteristics of the Alkaloid and Its Salts. The free alkaloid, as stated before, crystallizes from chloroform in reddish-brown needles. Its chloroformic solution is very dark in color, ranging from yellow, in very dilute solutions, to a dark reddish-brown, in saturated solutions. It is somewhat soluble in alcohol, very slightly soluble in water, almost insoluble in ether, benzol, and petroleum ether.

It dissolves in concentrated H_2SO_4 with reddish-brown color, the solution giving no change in color on adding a crystal of $K_2Cr_2O_7$. When heated, it fused together at about 220°, but did not melt even when heated to 280°, above which temperature it was not heated. At this temperature, it was in the form of a black mass.

The hydrochloride fused together at 310°, but was not completely melted at this temperature. It was not heated higher than this.

An aqueous solution of the hydrochloride gives the following reactions:

With $NaNO_3$ solution, a yellowish precipitate.
With Mayer's Reagent, a yellow precipitate.
With Wagner's Reagent, a reddish-brown precipitate.
With Tannic Acid solution, a yellowish precipitate.
With Picric Acid solution, a yellow precipitate.

Yellow amorphous precipitates with potassium ferrocyanide and potassium ferricyanide solutions.

With $KCrO_4$ solution, a yellow precipitate.
With platinic chloride, a yellowish-white precipitate.

The hydrochloride may be precipitated from its solution by adding a small amount of sodium chloride solution.

The solution of hydrochloride gives no precipitate with phosphoric, sulphuric, oxalic, tartaric or citric acids.

The percentage of sempervirine in gelsemium is small. About 0.8 gram was obtained from the 25 grams of alkaloidal material which represented the alkaloids from 25 pounds of the drug.

F.—The Filtrate from the Sempervirine Nitrate, together with further acid washings of the $CHCl_3$ solution which did not give a precipitate with $NaNO_3$, were extracted with several successive portions of benzol, using in all about three times the volume of the acid solution. This removed the greater part of the gelsemic acid. The acid liquid was then extracted with several successive portions of $CHCl_3$, using in all about four times the volume of the acid liquid. The chloroform removed the remainder of the gelsemic acid, the remainder of the sempervirine as the hydrochloride and also would undoubtedly remove at least traces of the hydrochlorides of all other alkaloids present, since such a large amount of $CHCl_3$ was used.

G.—The Chloroformic Solution. The chloroformic solution was filtered and the greater part of the $CHCl_3$ distilled off. The remainder was shaken with several small portions of water, until the hydrochlorides of the alkaloids were removed. The residual $CHCl_3$ was rejected. The aqueous solution was evaporated with a small amount of sand (sand being added to facilitate subsequent extraction) at a low temperature to dryness, the residue transferred to a flask and extracted with several successive portions of acetone. Sempervirine hydrochloride, which had not been precipitated with $NaNO_3$, is left with the sand from which it was extracted with alcohol.

The acetone solution was evaporated to dryness at a low temperature and the residue dissolved in water. (Not all of it went into solution.) The filtered solution was treated, drop by drop, with a saturated solution of NaCl. A small quantity of a yellow alkaloidal precipitate was obtained at first, the precipitate gradually becoming whiter. The first portion of the precipitate

was filtered off and the succeeding precipitate produced by NaCl was collected separately. This latter precipitate was dried on porous plate and dissolved in alcohol from which it separated in crystalline form. The crystals were almost colorless, slowly soluble in water and melted at 282° with decomposition.

A small amount of alkaloid hydrochloride remained in solution after saturating with NaCl. The solution was made alkaline with NH_4OH and the alkaloid extracted with $CHCl_3$. On evaporating the $CHCl_3$, a bitter amorphous residue with a pyridine-like odor was obtained. All of these alkaloidal products obtained here were very small in quantity.

H.—Acid Solution which had been Extracted with Benzol and $CHCl_3$. This acid solution was exactly neutralized to litmus with NaOH solution and evaporated at a low temperature. The dry residue was extracted with alcohol leaving behind NaCl and gelsemine hydrochloride, while a small quantity of gelsemine hydrochoride, and all of the gelseminine hydrochloride was dissolved. To remove as much as possible of the gelsemine hydrochloride, part of the alcohol was removed by distillation and the concentrated solution allowed to stand for a few days when a small amount of gelsemine hydrochloride separated out. The solution was filtered off and evaporated with sand at a low temperature. The residue was transferred to a flask and extracted with several successive portions of acetone. A small amount of alkaloidal hydrochloride remained in residue and was not extracted by the acetone.

This residue was dissolved with alcohol and, on evaporation of the alcohol, gave a crystalline hydrochloride. The hydrochloride was dissolved in water and the solution filtered. To the filtrate a saturated solution of NaCl was added which gave a small amount of yellow precipitate. This precipitate was not examined further. On adding more NaCl solution and even after saturating with NaCl, there was no further precipitate, but the hydrochloride of an alkaloid remained in solution. This was precipitated with NH_4OH, the precipitate collected, washed with water and dried. It was redissolved in alcohol, but did not separate in a crystalline form (labeled Alk. A.).

I.—Gelseminine. The acetone solution obtained in "H" was evaporated, the residue dissolved in water and the solution filtered. On adding to the solution a saturated solution of NaCl, there was produced with a small amount of NaCl solution a dark colored precipitate, while on adding more NaCl solution to the filtrate from this a further lighter colored precipitate was formed. After saturating with NaCl, there still remained some alkaloidal hydrochloride in the solution (precipitates labeled, first, *Precipitate with NaCl* and, second, *Precipitate with NaCl,* respectively).

It seems that gelseminine must consist of at least two alkaloids. This is indicated First, by the fact that the hydrochloride of one is more easily salted out from its solution than that of the other, the hydrochloride first separating out being much darker in color than that separated on further addition of NaCl solution. Second, if the free alkaloid be dissolved in a small amount of alcohol and ether* added, a very dark colored alkaloid is precipitated out

*The ether solution corresponds to what we have employed in physiological tests as gelseminine.

while one much lighter in color remains in solution. Neither of these two methods of fractional precipitation gives a sharp separation into two constituents and it could probably only be determined by an ultimate analysis of the two products or by comparison of physiological effects whether these are really two different products. The physiological method would apparently be the better test to use since it would require less material and less work.

SUGGESTED METHOD FOR SEPARATION OF ALKALOIDS OF GELSEMIUM.

A.—Extract Gelsemium with 90 percent alcohol. Evaporate at a low temperature. Add water to precipitate resinous matter and filter. Wash residue with distilled water. Make the combined filtrate and washings alkaline with NH_4OH and extract completely with $CHCl_3$. Filter the combined $CHCl_3$ extracts and distil off the greater part of the $CHCl_3$. Extract the alkaloid from $CHCl_3$ residue with one percent HCl. To the acid washings add a saturated solution of $NaNO_3$ until no further precipitate is formed, avoiding an excess of $NaNO_3$.

NOTES—The saturated solution of $NaNO_3$ need not be added to the whole of the acid washings. Each successive acid washing may be tested with $NaNO_3$ solution until a washing is obtained which does not give a precipitate with $NaNO_3$. The $NaNO_3$ solution need then be added only to the combined washings obtained previous to this washing which does not give a precipitate with $NaNO_3$ solution.

B.—(1) *Purification of the Sempervirine Nitrate.* Wash the nitrate on the filter paper with a 5 percent solution of $NaNO_3$ until free from the mother liquor. Wash the precipitate then drop by drop with distilled water until practically free from $NaNO_3$ solution, rejecting the first portions of the washings, but collecting the succeeding portions. (This collection of succeeding portions is necessary since, as soon as the greater part of the $NaNO_3$ solution is removed, the sempervirine nitrate dissolves to a slight extent). When washed free from $NaNO_3$, transfer the sempervirine nitrate to a beaker and heat with distilled water on a boiling water bath until solution is effected. Filter, add the portion of the washings collected above, and also a few drops of a saturated solution of $NaNO_3$. Filter off the precipitated sempervirine nitrate and proceed as before. Repeat this three or four times. Finally dry the sempervirine nitrate on porous plate and then recrystallize from hot alcohol.

(2) *Preparation of Free Alkaloid from Nitrate.* Dissolve the nitrate in hot water and add NH_4OH to the solution. Extract the alkaloid with $CHCl_3$. Distil off the major portion of the $CHCl_3$ and allow to crystallize.

(3) *Preparation of Hydrochloride from the Alkaloid.* Dissolve the free alkaloid in one percent HCl, filter and add a saturated solution of NaCl. Proceed as under B (1) except that an NaCl solution is used instead of an $NaNO_3$ solution.

C.—Proceed with the extraction as directed under A until the alkaloids are extracted. Combine these washings with the filtrate from the sempervirine nitrate and extract with several portions of benzol, using in all a quantity of benzol equal to about three times the volume of the acid washings. This removes the greater part of the gelsemic acid. Distil off benzol to get acid. Then extract the acid liquid with several portions of $CHCl_3$ using in all

three or four times the volume of the former. This will remove the remainder of the sempervirine as the hydrochloride, together with some other alkaloidal material, also any remaining gelsemic acid. Distil off the greater part of the $CHCl_3$ and shake the residue with several portions of distilled water. This will transfer the hydrochlorides of the alkaloids to the aqueous liquid. Mix the combined aqueous extracts with some sand and evaporate at a low temperature. Extract the residue with acetone. Sempervirine hydrochloride is left behind with the sand from which it may be extracted with alcohol.

The acetone solution may be further treated as above.

D.—The acid liquid which has been extracted with benzol and $CHCl_3$ is made just neutral with NaOH and evaporated to dryness at a low temperature. The residue is treated with alcohol in small amounts which leaves behind NaCl and gelsemine hydrochloride. In solution we have a small amount of gelsemine hydrochloride together with gelseminine hydrochloride and any other alcohol soluble hydrochlorides. This alcoholic solution is evaporated to a small volume and allowed to stand for some time when the major portion of the gelsemine hydrochloride will separate out. Filter, mix the filtrate with sand and evaporate at a low temperature. Extract the residue with acetone. The so-called gelseminine hydrochloride goes into solution while a small amount of alkaloidal material remains in the sand from which it may be extracted with alcohol.

Physiological Test: Thus far no complete pharmacological data has been obtained concerning this alkaloid. It may be stated, however, that 1 cc. of the solution of the hydrochloride containing 0.001 gm. of the salt killed a guinea pig weighing 90 gms. in 48 hours. A mouse weighing about 20 gms. with the same dose died in the same time, the salt having no *immediate* toxic effect.

NOTE: For the crude material containing the mixed alkaloids we are indebted to Messrs. Eli Lilly and Company and for crude and authentic material in working up former papers we are indebted to J. U. Lloyd, Parke, Davis and Company and H. K. Mulford Company. For their generous supply of material we desire to express our thanks.

Toxicity of Sempervirine: A report from the pharmacological laboratory of Parke, Davis & Co., gives the toxicity of Sempervirine as Minimum Lethal Dose = 0.00014 gm. per gram weight of frog. For this report the writer is indebted to Dr. E. M. Houghton.

LEUCOCYTIC EXTRACT—ITS PREPARATION AND USES.*

ARTHUR R. MEINHARD.

It is now a well established fact that one of the chief forms of protection of the animal body to bacterial invasion is through the action of certain of the white blood cells or the so-called phagocytic cells. These cells, which are easily able to wander through the walls of the blood vessels, are drawn by positive chemotactic action to the infected area and act by ingesting the trouble causing bacteria, destroying them and neutralizing their poisons. It is the purpose of this paper to dwell especially upon the nature of these poison neutralizing sub-

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