medicinal is concerned only with the maintenance of uniformly high potency in different lots of the same type of product.

CONCLUSIONS.

1. With the original ovarian follicular hormone ketohydroxyestrin (theelin), one hypodermic rat unit of activity is equivalent to five hypodermic mouse units.

2. With the benzoates of ketohydroxyestrin (theelin) and of dihydroxyestrin, the relationship of the rat and mouse units are found to be 5 to 1 and 1 to 1, respectively, rather than 1 to 5.

3. The desirability of stating the potency of the various estrogenic principles in the proper type of International Unit is pointed out.

---

## EXTRACTION STUDIES ON IPECAC.*[1]

BY SAMUEL W. GOLDSTEIN.

A number of studies on drug extraction have appeared in the literature dealing with the various factors involved in the process of percolation. With regard to the extraction of ipecac, the following menstrua have been used in the studies reported: Alcoholic, hydroalcoholic and acidified hydroalcoholic. Remington (1) pointed out the value of acetic acid as a solvent and as a menstruum for the extraction of drugs, and proposed a new class of galenicals, "acetracts," prepared with acetic acid of various concentrations. In a second paper (2) he reported on the preparation of "acetract of ipecac" with 60% acetic acid as the menstruum, and also that when weaker strengths of acetic acid were used the preparation gelatinized on standing. Roberts (3) recommended that syrup of ipecac be made from a vinegar of ipecac, like syrup of squill.[2] He stated: "acetic acid is a good solvent of the emetic properties of ipecac root." Wayne (4) prepared an acetic syrup of ipecac by macerating the drug in dilute acetic acid for 7 days, expressing and filtering, and then proceeding in the manner prescribed for syrups. Procter (5) prepared a water- and syrup-miscible fluidextract of ipecac by extracting with alcohol, distilling off the alcohol, and pouring the syrupy residue remaining into water. Breddin (6) prepared a fluidextract of ipecac by the process of diacolation (a modified fractional percolation). Other investigators (7, 8, 9, 10) have studied the effects of aqueous, hydroalcoholic and acidified hydroalcoholic menstrua on the extraction of ipecac, using the processes of maceration and infusion. Gstirner (11) studied the extraction of ipecac with water and with acidified menstrua, using hydrochloric and citric acids. He presented the results of other workers in a series of tables. Steiger (12) found, by maceration, that stronger alcoholic menstrua extracted the alkaloids and total solids more readily from finely powdered ipecac than from the coarser powders, but more dilute alcohol gave better results with a coarser powder. Bull (13) found that in the extraction of cinchona and belladonna a moderately fine powder gave the best results. Husa and Huyck (14), working with belladonna, found that within

---

* Scientific Section, A. PH. A., Dallas meeting, 1936.

[1] From the research laboratories of the School of Pharmacy of the University of Maryland.

[2] The Syrup of Squill official in 1858 was prepared by dissolving the sugar in the Vinegar of Squill, with the aid of gentle heat, and straining the solution while hot.

the limits of No. 20 and No. 80 powders, the fineness of the powder is of minor importance.

The present paper deals with the problem mainly as it concerns the manufacturing pharmacist, who wishes to obtain the important constituents of ipecac in such a form that they may be incorporated directly in galenical preparations, rather than the complete exhaustion of the crude drug beyond the point where the cost is prohibitive.

## EXPERIMENTAL.

The ipecac used in this study met all the requirements of the U. S. P. X (1.76% alkaloids) and was in the form of a No. 20 powder. The drug was packed with approximately equal pressures in similar, slightly conical, glass percolators. In each case a 500-Gm. sample of the mixed lot of drug was used, and 250 cc. of the indicated menstruum were added to moisten the powder. Bull's (13) modification of Lenton's apparatus for the recovery of desired volumes of percolate was used, and the percolation was carried out at the rate of 12 drops per minute except where otherwise noted. In all cases, a first fraction of 500 cc. of percolate was collected and fractions of 250 cc. of percolate were collected thereafter. The percolation was continuous in all cases except the third experiment in the series in which acetic acid was used as menstruum. The menstrua used were those of the U. S. P. X, U. S. P. XI and acetic acid (9%), which has been found to be the lowest concentration of acetic acid that will prevent mold growth in the percolator.

**U. S. P. X Menstrua.** *Experiment 1.*—The drug was moistened with part of the first menstruum (alcohol 2, water 2, diluted hydrochloric acid 1 volume) and kept in a closed vessel for 24 hours, then it was packed and macerated, with the remainder of Menstruum I and a sufficient quantity of Menstruum II (alcohol 2, water 3 volumes), for 96 hours before starting the percolation.

*Experiment 2.*—The drug was moistened with Menstruum I, allowed to stand for 1 hour, then packed and, after adding the remainder of Menstruum I and a sufficient quantity of Menstruum II, allowed to macerate for 72 hours.

*Experiment 3.*—The drug was moistened with Menstruum I, allowed to stand for 1 hour, then packed and, after adding the remainder of Menstruum I and a sufficient quantity of Menstruum II, allowed to macerate for 48 hours.

**U. S. P. XI Menstruum.** In both experiments conducted with this menstruum (alcohol 3, water 1 volume) the U. S. P. XI procedure was followed.

**Acetic Acid (9%).** *Experiment 1.*—The drug was moistened and kept in a closed vessel for 24 hours, then it was packed and, after adding a sufficient quantity of menstruum, allowed to macerate for 48 hours before starting the percolation.

*Experiment 2.*—The drug was moistened, kept in a closed vessel for 2 hours, then it was packed and, after adding a sufficient quantity of menstruum, allowed to macerate for 72 hours.

*Experiment 3.*—The procedure followed was the same as in Experiment 2, but the percolation, in Experiment 3, was stopped after each fraction of percolate was collected and the drug was allowed to macerate for 72 hours before continuing the percolation.

Each fraction of percolate was assayed for ether-soluble alkaloids by the method in the U. S. P. XI, and for total solids by drying a measured volume of percolate to constant weight in an oven at 100° C.

The percentage (w/v) of alkaloids in each fraction of percolate, and the percentage (w/w) of alkaloids extracted in the first 1000 cc. of percolate and, in those instances where the percolation was continued, in the 1500 cc. of percolate are given in Table I.

The results in Table I show that the U. S. P. X menstrua, after allowing the moistened drug to stand for 24 hours before packing and then allowing a 96-hour period of maceration, extracted a greater proportion of alkaloids than when the macerating periods were reduced to 1 hour before and 72 hours after packing, respectively, especially in the first fraction of percolate. When the macerating periods were reduced to 1 hour and 48 hours, respectively, the proportion of alkaloids in the first fraction of percolate dropped considerably. However, as the percolation continued, the total amount of alkaloids extracted approached a more nearly constant value, particularly in the latter two experiments.

TABLE I.—PERCENTAGE (W/V) OF ALKALOIDS IN FRACTIONS OF PERCOLATE.

| Menstrua. | Fractions of Percolate. 1. | 2. | 3. | 4. | 5. | Alkaloids from 500 Gm. of Drug in % w/w. 1st 1000 Cc. of Percolate. | 1500 Cc. of Percolate. |
|---|---|---|---|---|---|---|---|
| U. S. P. X | 1.18 | 0.25 | 0.18 | .. | .. | 1.40 | .. |
| | 1.01 | 0.27 | 0.17 | .. | .. | 1.23 | .. |
| | 0.83 | 0.40 | 0.33 | 0.15 | 0.15 | 1.20 | 1.35 |
| U. S. P. XI | 1.11 | 0.30 | 0.14 | .. | .. | 1.33 | .. |
| | 1.07 | 0.40 | 0.17 | 0.03 | 0.05 | 1.36 | 1.40 |
| Acetic Acid (9%) | 1.07 | 0.29 | 0.10 | .. | .. | 1.27 | .. |
| | 1.18 | 0.18 | 0.15 | 0.04 | 0.03 | 1.35 | 1.39 |
| | 0.96 | 0.57 | 0.41 | 0.09 | 0.04 | 1.44 | 1.51 |

The length of the periods of maceration before and after packing were the same in both experiments carried out with U. S. P. XI menstruum, and the results are in fairly close agreement.

The figures resulting from the experiments with acetic acid (9%) indicate that maceration after packing exerts a greater influence on the extraction of the alkaloids than does the maceration before packing; for in the first two experiments the total lengths of time for maceration were about the same, but in the second experiment the time before packing was 22 hours shorter and the time after packing was correspondingly longer than in the first experiment. The percentage of alkaloids obtained in the first percolate of the second experiment was 10% greater than the percentage of alkaloids obtained in the corresponding percolate in the first experiment.

The rate at which percolation was allowed to proceed had a definite effect on the rate of extraction of the alkaloids as may be seen from the results of the first fractions of percolate which were obtained by percolating at the rate of 8 drops per minute in the first two experiments and at the rate of 16 drops per minute in the third experiment. In the third experiment, interrupting the percolation and allowing the drug to macerate for 72 hours greatly increased the yield of alkaloids in each of the succeeding fractions, as compared to the corresponding fractions obtained in the other experiments, and resulted in the extraction of a higher total amount of alkaloids than was obtained in any of the other experiments.

The percentage (w/v) of total solids extracted in each fraction of percolate, and the percentage (w/w) of total solids extracted in the first 1000 cc. of percolate and in 1500 cc. of percolate are given in Table II.

TABLE II.—PERCENTAGE (W/V) OF TOTAL SOLIDS IN FRACTIONS OF PERCOLATE.

| Menstrua. | Fractions of Percolate. 1. | 2. | 3. | 4. | 5. | Total Solids from 500 Gm. of Drug in % w/w. 1st 1000 Cc. of Percolate. | 1500 Cc. of Percolate. |
|---|---|---|---|---|---|---|---|
| U. S. P. X | 19.74 | 4.21 | 1.77 | .. | .. | 22.73 | ... |
| | 16.34 | 4.78 | 3.03 | .. | .. | 20.25 | ... |
| | 12.33 | 6.60 | 5.75 | 3.31 | 2.37 | 18.51 | 21.35 |
| U. S. P. XI | 15.47 | 4.94 | 2.02 | .. | .. | 18.95 | ... |
| | 14.85 | 6.45 | 2.62 | 1.19 | 0.94 | 19.38 | 20.45 |
| Acetic Acid (9%) | 19.58 | 5.20 | 2.73 | .. | .. | 23.55 | ... |
| | 21.65 | 3.63 | 1.60 | 0.76 | 0.54 | 24.27 | 24.91 |
| | 16.81 | 10.97 | 5.49 | 1.12 | 0.61 | 25.04 | 25.91 |

The figures in Table II show that the yield of total solids in the first fraction of percolate in the experiments with U. S. P. X and U. S. P. XI menstrua varied directly with the length of time allotted to the periods of maceration before and after packing, with the maceration after packing having the more pronounced effect. Similar results were obtained in the first two experiments with acetic acid; for, although the total times of maceration were approximately equal, a shorter preliminary and correspondingly longer secondary maceration was allowed in the second experiment. In the first two experiments with acetic acid the percolation proceeded at about 8 drops per minute. The third experiment with the same menstruum, after maceration periods similar to those in the second experiment, was carried out at the rate of 16 drops per minute and gave the lowest yield of extracted matter in the first fraction of percolate. However, as the

percolation continued, the amount of total solids obtained approached a fairly constant value for each menstruum. The acetic acid extracted more of the total solids in every case than was extracted by either of the other two menstrua.

The figures for the ratio of alkaloids to other solids, *i. e.*, percentage of total solids minus percentage of alkaloids, are given in Table III, expressed as parts of alkaloids per 100 parts of other solids.

TABLE III.—RATIO OF ALKALOIDS TO 100 PARTS OF OTHER SOLIDS.

| Menstrua. | Fractions of Percolate. 1. | 2. | 3. | 4. | 5. | Ratio Obtained from Total Extractives. 1st 1000 Cc. | 1500 Cc. |
|---|---|---|---|---|---|---|---|
| U. S. P. X | 6.36 | 6.31 | 11.32 | .. | .. | 6.56 | .. |
| | 6.59 | 5.99 | 5.94 | .. | .. | 6.46 | .. |
| | 7.21 | 6.45 | 6.09 | 4.74 | 6.75 | 6.93 | 6.75 |
| U. S. P. XI | 7.74 | 6.47 | 7.45 | .. | .. | 7.60 | .. |
| | 7.76 | 6.61 | 6.90 | 2.58 | 5.62 | 7.54 | 7.35 |
| Acetic Acid (9%) | 5.78 | 5.91 | 3.80 | .. | .. | 5.70 | .. |
| | 5.76 | 5.22 | 10.34 | 5.55 | 5.88 | 5.89 | 5.91 |
| | 6.05 | 5.48 | 8.07 | 8.73 | 7.02 | 6.10 | 6.19 |

The figures in Table III indicate that the different menstrua used in continuous percolation do not extract the alkaloids and other solids from ipecac at the same rate. For each menstruum the rates appear to be roughly parallel, but, since the factors affecting extraction were varied, no general conclusion can be drawn. When interrupted percolation was carried out with acetic acid (9%) as menstruum, the total amount of alkaloids extracted increased proportionally more than the total amount of other solids as indicated by the higher ratio. The ratios obtained from the total extractives of alkaloids and other solids show that the U. S. P. XI menstruum extracted the smallest amount of other solids, whereas it extracted the alkaloids fairly well; and that acetic acid (9%) extracted the largest amount of other solids regardless of the manner of percolation and the amount of alkaloids extracted at the same time.

### LARGE SCALE EXTRACTIONS.

**U. S. P. IX Menstrua and Acetic Acid** (9%).—Fifty pounds (22.72 Kg.) of the same lot of No. 20 powdered ipecac that was used in the earlier experiments were extracted in a cylindrical earthenware percolator. The drug was moistened with Menstruum I of the U. S. P. IX and kept in a closed vessel over night, then it was packed and macerated with the remainder of the Menstruum I and a sufficient quantity of Menstruum II for 96 hours before starting the percolation, which was carried out at the rate of 9 drops per minute. The menstrua given in the U. S. P. IX were the same as those given in the U. S. P. X for Fluidextract of Ipecac, with the exception that two-thirds as much of Menstruum I (alcohol 2, water 2, diluted hydrochloric acid 1 vol.) was used in the earlier procedure. After the first fraction of percolate (50 pints) was collected, the addition of the hydroalcoholic menstruum was discontinued and acetic acid (9%) was added thereafter. The last two fractions of percolate were collected at the rate of 8 drops per minute. Total elapsed time for the extraction was 66 days.

**Acetic Acid** (9%).—Fifty pounds of the No. 20 powdered ipecac were moistened with acetic acid (9%) and kept in a closed vessel over night, then it was packed in a cylindrical earthenware percolator and, after adding a sufficient quantity of menstruum, macerated for 144 hours before starting the percolation, which was carried out at the rate of 13 drops per minute for the first two fractions of percolate and at the rate of 25 drops per minute for the last fraction of percolate. After each of the first two fractions of percolate were collected, the percolation was stopped and the drug was macerated for 72 hours. Total elapsed time for the extraction was 54 days.

In both experiments fractions of 50 pints (23.57 liters), 22.5 pints (10.61 liters) and 27.5 pints (12.97 liters) were collected.

The percentage (w/v) of alkaloids in each fraction of percolate, and the percentage (w/w) of alkaloids extracted from the drug are given in Table IV.

TABLE IV.—PERCENTAGE (W/V) OF ALKALOIDS IN FRACTIONS OF PERCOLATE.

| Menstrua. | Fractions of Percolate. 1. | 2. | 3. | Alkaloids from 50 Lbs. of Drug in % w/w |
|---|---|---|---|---|
| U. S. P. IX and Acetic Acid (9%) | 1.02 | 0.37 | 0.07 | 1.27 |
| Acetic Acid (9%) | 1.21 | 0.36 | 0.10 | 1.48 |

The results in Table IV show that interrupted percolation with acetic acid (9%) extracted a higher percentage of alkaloids than was extracted by the menstrua given in the U. S. P. IX for the first fraction, and acetic acid (9%) for the second and third fractions of percolate with continuous percolation. The percentage of alkaloids extracted in the second experiment was 16.5% greater than the percentage of alkaloids extracted in the first experiment. Furthermore, it should be noted that although, in the second experiment, the preliminary maceration was 2 days longer and the percolation was interrupted twice for periods of 3 days, the total elapsed time for the extraction was 54 days as compared with 66 days for the extraction in the first experiment.

The percentage (w/v) of total solids in each fraction of percolate, and the percentage (w/w) of total solids extracted from the drug are given in Table V.

TABLE V.—PERCENTAGE (W/V) OF TOTAL SOLIDS IN FRACTIONS OF PERCOLATE.

| Menstrua. | Fractions of Percolate. 1. | 2. | 3. | Total Solids from 50 Lbs. of Drug in % w/w. |
|---|---|---|---|---|
| U. S. P. IX and Acetic Acid (9%) | 17.47 | 5.33 | 1.90 | 21.70 |
| Acetic Acid (9%) | 21.25 | 4.67 | 1.62 | 25.20 |

The figures in Table V show that acetic acid (9%) extracted more of the total solids than was extracted by the menstrua used in the first experiment. The percentage of total solids extracted in the second experiment was 16.1% greater than the percentage of total solids extracted in the first experiment.

The figures for the ratio of alkaloids to other solids, expressed as parts of alkaloid per 100 parts of other solids, are given in Table VI.

TABLE VI.—RATIO OF ALKALOIDS TO 100 PARTS OF OTHER SOLIDS.

| Menstrua. | Fractions of Percolate. 1. | 2. | 3. | Ratio Obtained from Total Extractives from 50 Lbs. of Drug. |
|---|---|---|---|---|
| U. S. P. IX and Acetic Acid (9%) | 6.20 | 7.46 | 3.82 | 6.22 |
| Acetic Acid (9%) | 6.03 | 8.35 | 6.58 | 6.23 |

The final figures in Table VI show that the relative increases in the percentages of alkaloids and total solids extracted in the second experiment, as compared with the percentages obtained in the first experiment, were approximately equal. This was found to be true; for the increase in the percentage of alkaloids extracted was 16.5%, while the increase in the percentage of total solids extracted was 16.1%.

## DISCUSSION OF RESULTS.

The results obtained in this investigation show that the hydroalcoholic menstruum adopted in the U. S. P. XI for the extraction of ipecac in the preparation of the fluidextract has been well chosen; inasmuch as it removes the alkaloids quite readily, and, at the same time, extracts less of the other solids than do the other menstrua studied. The efficacy of shortening the time of preliminary maceration before packing and increasing the time of maceration after packing, as directed in the U. S. P. XI has been definitely demonstrated. Procter (15) and, more recently, Scoville (16) have contended that moistening the drug before packing is of value

only in cases where the powdered drug swells on addition of the menstruum used. This has been substantiated by Husa and Yates (17), working with belladonna root, and is further confirmed by the present results with ipecac.

Husa and Yates (17), working with belladonna root (menstr.: alcohol 5, water 1 vol.), found that maceration after packing was of no appreciable value in promoting rapid extraction of alkaloids. Husa and Huyck (18), working with belladonna root (Menstruum: alcohol 5, water 1 vol.), yellow cinchona (Menstruum: glycerin 1, hydrochloric acid 1, alcohol 8 volumes), and nux vomica (Menstruum I: acetic acid 100 cc., water 150 cc., alcohol 750 cc.; Menstruum II: alcohol 3, water 1 volume), found that maceration after packing caused a slight increase in efficiency of extraction. The experiments reported here, in which the ipecac was extracted with the U. S. P. X menstrua, gave the following results: When the drug was macerated for 24 hours before packing and 96 hours after packing, the percentage of alkaloids in the first percolate was 1.18%, which was 16.8% greater than the percentage of alkaloids (1.01%) in the first percolate obtained in the second experiment (macerated for 1 hour before and 72 hours after packing). In the third experiment, the first percolate, obtained after macerating for 1 hour before packing and for 48 hours after packing, contained 0.83 per cent of alkaloids, which was 17.8% less than the percentage of alkaloids in the first percolate obtained in the second experiment.

The results of the experiments carried out with acetic acid (9%) as menstruum also indicate that the length of the period of maceration after packing the drug directly influences the efficiency of extraction.

Although a higher yield of alkaloid was obtained in the first portion of percolate when the drug was macerated for a longer period before percolation, this advantage practically disappeared as the percolation continued. However, it is undoubtedly an advantage to have the desired principles of the drug extracted with as little menstruum as possible, especially in large scale operations.

Extraction of ipecac with acetic acid (9%) was more efficient when percolation was carried out at a rate of 8 drops per minute than when the rate was increased to 16 drops per minute. Interrupted percolation, in which the menstruum is allowed to become more nearly saturated, by maceration, with respect to the substances being extracted, is notably helpful in attaining the above-mentioned goal in the extraction of ipecac with acetic acid. Husa and Yates (17) found that this did not hold in the case of belladonna root extracted with the official menstruum (alcohol 5, water 1 volume).

Bull (13) found that, in the extraction of cinchona with 80% alcohol, the relative percentage of alkaloids to other solids increased in successive fractions of percolate. He found the opposite to be true in the extraction of belladonna root with 90% alcohol. In the present work, the relative percentage remained roughly the same for each menstruum, indicating that in the case of ipecac, as long as the same menstruum is used, variations in certain of the other factors influencing extraction affect the removal of the alkaloids and other solids to relatively the same extent.

The results obtained in the extraction of 50-pound batches of powdered ipecac are in agreement with the results of the experiments with 500-Gm. portions of drug and emphasize the applicability of acetic acid (9%) as a menstruum for the extraction of ipecac.

We have no doubt that general statements, as to the predicted behavior of different drugs with various menstrua, cannot be of any value in the light of our present knowledge; and, that the effects of varying the factors involved in any set of conditions pertaining to drug extraction must be determined experimentally.

## CONCLUSIONS.

1. The menstruum and procedure given in the U. S. P. XI for the extraction of ipecac in the preparation of the fluidextract have been proved to be well chosen.

2. When similar methods were used, the U. S. P. XI menstruum extracted a higher percentage of alkaloids and a lower percentage of other solids than were extracted by the U. S. P. X menstrua or by acetic acid (9%).

3. Interrupted percolation with acetic acid (9%) yielded a higher percentage of alkaloids than was obtained by continuous percolation with either of the other two menstrua used. At the same time the percentage of total solids extracted by the acetic acid was increased only slightly over the amount extracted by the same menstruum by continuous percolation.

4. Although the maceration before packing the drug need be only long enough to allow swelling to occur, the length of the period of maceration after packing is important, especially if one wishes to obtain as much of the alkaloids as possible in the first fractions of percolate.

5. In manufacturing or in other instances, in which a comparatively large amount of total solids is unobjectionable, extraction with acetic acid (9%) is satisfactory, and results in considerable reduction in the cost of the extraction of ipecac.

Further investigations are being carried out on ipecac and other drugs.

The author is indebted to Mr. Sidney Hollander for suggesting the problem, and to the Maryland Pharmaceutical Co. for supplying the drug and for the use of its extraction facilities.

The author also wishes to express his appreciation to Dr. A. G. DuMez for his interest in the work.

## REFERENCES.

(1) Remington, J. P., *Am. J. Pharm.*, 69, 121 (1897).
(2) Remington, J. P., *Ibid.*, 70, 543 (1898).
(3) Roberts, J., *Journal and Transactions, Md. College of Pharmacy*, 1, 30 (1858).
(4) Wayne, E. S., *Druggist* (July 1861); through *Journal and Transactions, Md. College of Pharmacy*, 2, 121 (1861).
(5) Procter, W., Jr., *Ibid.*, 2, 150 (1861).
(6) Breddin, H., *Pharm. Ztg.*, 77, 1153 (1932).
(7) Kogan, G., *Pharm. Zentralh.*, 70, 600 (1929).
(8) Bruch, G., *Apoth.-Ztg.*, 44, 612 (1929).
(9) Bauer, K. H., and Haber, K., *Pharm. Zentralh.*, 71, 513 (1930).
(10) Steudel, H., *Pharm. Ztg.*, 75, 1450 (1930).
(11) Gstirner, F., *Pharm. Zentralh.*, 76, 421 and 437 (1935).
(12) Steiger, K., *Pharm. Acta Helv.*, 10, 59 (1935).
(13) Bull, A. W., *Quart. J. Pharm. Pharmacol.*, 8, 378 (1935).
(14) Husa, W. J., and Huyck, C. L., JOUR. A. PH. A., 24, 446 (1935).
(15) Procter, W., Jr., *Am. J. Pharm.*, 31, 317 (1859).
(16) Scoville, W. L., JOUR. A. PH. A., 21, 877 (1932).
(17) Husa, W. J., and Yates, S. B., *Ibid.*, 24, 538 (1935).
(18) Husa, W. J., and Huyck, C. L., *Ibid.*, 25, 311 (1936).