

3. The preference of International authorities for a lethal dose frog method for the bioassay of digitalis preparations and the adoption of such a method by the B. P. 1932 and the 1934 Canadian Regulations are important indications which are substantiated by the experimental data here presented.

4. Both the official method and the correction factor of the U. S. P. XI digitalis standard powder should be changed by interim revision so that the U. S. P. tincture of digitalis shall conform in potency to the International Standard for Tr. Digitalis.

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DRUG EXTRACTION. XIX. THE EFFECT OF PRESSURE AND VACUUM ON EFFICIENCY OF EXTRACTION.*¹

BY WILLIAM J. HUSA² AND GEORGE R. JONES.

For more than one hundred and twenty years various types of drug extraction processes have been used in which pressure and vacuum have been employed. In the present paper the methods used in the past are summarized and classified and a report is given covering the experimental work of the present investigation in which a study was made of the effect of vacuum on the efficiency of extraction of belladonna root.

HISTORICAL REVIEW.

Hydrostatic Pressure of Liquid.—In 1816 Count Real devised an extraction apparatus in which a long pipe, frequently 50 or 60 feet in length, extended straight upward from the vessel containing the material to be extracted (1). The hydrostatic pressure of the liquid was presumed to be favorable to extraction. To shorten the column of liquid, Real devised a means of using a column of mercury to exert its hydrostatic pressure on the menstruum above the drug. Various modifications of the Real apparatus were made by Geiger (2), Dobereiner (3), Brandes (4), Wurzer (5) and Beindorf (6).

Air Pressure Applied on the Surface of the Menstruum.—In 1817 Semmelbauer applied pressure to the surface of the menstruum by use of an air compression machine (7). Pressure pumps were also used in percolation by Schubart (8), Payen (9) and Beral (10). In later years compressed air was used in percolation by Signoret (11), McPherson (12), Hinsdale (13), Phillips (14), Hoseason (15) and Lenz (16). Romershausen used steam pressure to force hot water through the drug

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(17). Marpmann, in 1908, described an apparatus in which pressure was obtained in an atmosphere of carbon dioxide or nitrogen, thus retarding deterioration of the drug; he also evacuated the receiving vessel (18).

Vacuum Applied to the Receiver, Percolator or Macerating Vessel.—One way of applying air pressure to the surface of the menstruum in percolation is to evacuate the receiving vessel. In 1818 Kastner used a vacuum pump for evacuating the receiver in a percolation process (19). Within the next several years vacuum pumps were used to evacuate the receiver by others, including Romershausen (17), Beindorf (6) and Beral (20). Martenstein, in 1828, obtained a partial vacuum by filling the receiver with steam and then placing it in water to condense the steam (21). In later years evacuation of the receiver was used by Vielguth and Nentwich (22), Maben (23), Platt (24), Hostelley (25) and Rogers (26).

The use of vacuum to increase the penetration of menstruum into the drug was suggested in 1869 by Duffield (27). Duffield stated that more perfect maceration could be obtained if the ground drug was placed in a strong cylinder; the air pumped out and the requisite amount of menstruum admitted. He stated that the pores of the comminuted drug gave up the air enclosed in them and when the menstruum was allowed to flow in it was forced into the pores by pressure of the air outside.

In 1882 Fairthorne (28) used a process of making fluidextracts which included vacuum maceration, followed by percolation into an evacuated receiver and the finishing of the operation by forcing compressed air into the percolator. A percolator of this type had been patented in 1870 by Merrell (29). A similar apparatus was later used by Thomson (30).

Extraction Apparatus Based on the Soxhlet Extractor.—For many years extraction devices based on the principle of the Soxhlet extractor have been used on a factory scale in the United States. By continuously evaporating the percolate and returning the recovered menstruum to the percolator several advantages are obtained. In 1918 Beard (31) described a continuous percolation process under reduced pressure based on the principle of the Soxhlet extractor; heat and suction were carefully regulated so that the liquid would boil between 35° and 40° C. A similar apparatus was later used by others (32).

Mulcolation.—In 1934 Kessler's process of making fluidextracts by mulcolation was described (33). Mulcolation is essentially a type of fractional percolation employing an evacuated receiver.

Evacolation.—In 1935 Kessler (34) described the evacolation process for making fluidextracts. Evacolation consists essentially of a percolation process in a single cylindrical tube, using vacuum maceration and an evacuated receiver, with careful regulation of the flow of menstruum into the percolator.

Mechanical Pressure Applied to the Menstruum.—Flowers (35) used a percolator which consisted of a metal cylinder with an air-tight piston. The rod of the piston was threaded, so that the piston could be screwed down tightly on the menstruum. The pressure obtained was estimated to be about 75 to 150 pounds.

Mechanical Pressure Applied to the Drug.—In 1825 Hänle used a percolator in which mechanical pressure was applied to the drug (36). An inner cylinder having a perforated bottom was slipped into the percolator and pressed against the drug. The inner cylinder was tied in place by means of strings, which were then wetted; the resulting contraction of the strings produced pressure on the drug. The men-

struum was then poured through the top of the inner cylinder and allowed to percolate.

Rosenwasser (37) secured mechanical pressure on the drug by packing it between porous diaphragms which were fixed in place; the swelling of the drug produced pressure. He also used the hydrostatic pressure of a column of liquid. Other workers who employed hydrostatic pressure of the liquid in conjunction with more or less mechanical pressure on the drug were Berry (38) and Cohn (39).

EXPERIMENTAL PART.

Materials Used.—To insure uniformity throughout the experimental work all of the drug used was taken from a thoroughly mixed 100-lb. portion of moderately coarsely powdered belladonna root. The results of numerous assays of the drug according to the U. S. P. XI method gave an average of 0.46 per cent of alkaloids. According to the U. S. P. XI method for drugs containing no constituents volatile at 100° C., the drug contained 13.9 per cent of moisture. The menstruum used was a mixture of four volumes of alcohol and one volume of distilled water as specified for fluidextract of belladonna root in the U. S. P. XI.

Methods of Analysis.—Alkaloids in the stronger percolates were determined by the U. S. P. XI assay process for fluidextract of belladonna root; in weak percolates the assay was made according to the U. S. P. XI method for tincture of belladonna. To determine total extractive, ten cc. of the liquid were evaporated to dryness on a water-bath and then heated in an oven at 105° C. until the difference between two successive weighings did not exceed ten mg.

Effect of Reduced Pressure on the Extraction of Belladonna Root by Maceration. Experiment 1.—Various writers have presumed that vacuum maceration increases the penetration of menstruum into the drug but apparently no quantitative determinations have been made on this point. Hence experiments were planned in order to determine the effect of vacuum on the proportion of menstruum imbibed by the powdered drug as well as to determine the effect of vacuum on the efficiency of extraction. This was done by using the centrifuge method previously developed by Husa and Magid (40).

Into each of four 50-cc. centrifuge tubes was placed a 2.50-Gm. portion of belladonna root. Using a vacuum pump, reduced pressure (pressure of 145 to 150 mm. of mercury) was applied for about two minutes and then 22.50 Gm. of menstruum were added to the contents of each tube by means of a burette which had previously been inserted through a hole in the stopper. Air was admitted and the mixture was allowed to stand fifteen minutes with stirring every five minutes. The tubes were then centrifuged for ten minutes, the liquid poured off and the tubes containing the wet marc were weighed. The liquids from the four tubes were combined and weighed.

Another series of tubes was handled in the same manner except that the vacuum was applied differently. In this case the menstruum was added to the drug at atmospheric pressure and the tubes placed in a desiccator which was partially evacuated (pressure of 140 mm. of mercury) for five minutes. Air was then admitted and the contents of the tubes were stirred. The tubes were evacuated in this manner three successive times.

As a control for both experiments another series of tubes was handled in the same manner except that reduced pressure was not used. The two experiments and control were carried out in duplicate.

The amount of dry marc was calculated as follows: (weight of drug taken for extraction) minus (weight of moisture in drug) minus (weight of total extractive in filtrate) = (weight of dry marc). The weight of menstruum imbibed by the marc was calculated as follows: (weight of wet marc) minus (weight of dry marc) = (weight of menstruum imbibed by the marc). The loss of menstruum during the process was determined by comparing the total weight of the materials used with the combined weight of the filtrate and wet marc.

Table I indicates that reduced pressure did not increase the efficiency of extraction of alkaloids or total extractive. Likewise reduced pressure had no effect on the proportion of menstruum imbibed by the marc.

TABLE I.—EFFECT OF REDUCED PRESSURE ON IMBIBITION AND EXTRACTION OF POWDERED BELLADONNA ROOT.

	Liquid Imbibed in Marc.	Weight in Gm. of			Total Extractive.	Total Alkaloids.
		Dry Marc.	Macerate.	Loss of Menstruum.		
A. Vacuum Applied before Adding Menstruum.						
	18.92	7.02	74.10	0.00	1.60	0.036
	18.74	7.06	74.10	0.11	1.55	0.037
Av.	18.83	7.04	74.10	0.06	1.57	0.037
B. Vacuum Applied after Adding Menstruum.						
	18.63	7.02	73.77	0.58	1.59	0.037
	18.57	7.06	73.84	0.54	1.55	0.036
Av.	18.60	7.04	73.80	0.56	1.57	0.037
C. Control (No Vacuum Used).						
	18.83	7.02	73.61	0.45	1.60	0.037
	18.85	7.02	73.88	0.25	1.59	0.037
Av.	18.84	7.02	73.75	0.35	1.59	0.037

Experiment 2.—Further tests were conducted in which the time of maceration under reduced pressure was increased to thirty minutes. The procedure followed in Experiment 1 was slightly modified. The entire 10.00 Gm. of belladonna root were placed in a wide-mouth bottle of about 200 cc. capacity. A two-hole rubber stopper was placed in the mouth of the bottle; one of the holes was fitted with a glass tube connected to the vacuum pump; a separatory funnel was inserted in the other. After applying a partial vacuum (pressure of 130 to 135 mm. of mercury) to the dry drug for thirty minutes, 90 Gm. of menstruum were added in rapid drops through the separatory funnel, ten minutes being required for the addition of the menstruum. During the addition of the menstruum the vacuum remained constant. Air was then allowed to enter the bottle and the mixture allowed to stand for thirty minutes with stirring every five minutes. The bottle containing the drug and menstruum was centrifuged ten minutes, the liquid decanted and the bottle containing the wet marc was weighed. The macerate was weighed and then assayed for total extractive and alkaloidal content. The experiment and control using no vacuum were carried out in duplicate.

Another experiment was conducted using the same procedure except that the reduced pressure was applied in a different manner. The menstruum was added to the drug at atmospheric pressure. The bottle was then placed in a desiccator and a partial vacuum (pressure of 130 to 135 mm. of mercury) was applied for five minutes, after which air was allowed to enter and the contents of the bottle thoroughly stirred. The bottle was evacuated in this manner six successive times. The experiment and a control using no vacuum were conducted in duplicate.

TABLE II.—EFFECT OF REDUCED PRESSURE ON IMBIBITION AND EXTRACTION OF POWDERED BELLADONNA ROOT.

	Liquid Imbibed in Marc.	Weight in Gm. of			Total Extractive.	Total Alkaloids.
		Dry Marc.	Macerate.	Loss of Menstruum.		
A. Vacuum Applied before Adding Menstruum.						
	18.86	7.02	73.77	0.36	1.60	0.039
	19.23	7.04	73.34	0.39	1.57	0.039
Av.	19.05	7.03	73.56	0.38	1.59	0.039
B. Control for Part A (No Vacuum Used).						
	18.95	7.01	73.65	0.40	1.60	0.038
	19.92	7.04	72.57	0.48	1.57	0.038
Av.	19.44	7.03	73.11	0.44	1.59	0.038

TABLE II.—(Continued from page 855.)

C. Vacuum Applied after Adding Menstruum.						
	19.29	7.03	72.92	0.77	1.58	0.039
	19.87	7.03	72.26	0.75	1.58	0.038
Av.	19.58	7.03	72.59	0.76	1.58	0.039
D. Control for Part C (No Vacuum Used).						
	19.55	7.02	72.96	0.48	1.60	0.039
	19.97	7.01	72.56	0.47	1.60	0.039
Av.	19.76	7.02	72.76	0.48	1.60	0.039

Table II confirms Table I in showing that vacuum does not increase imbibition of menstruum or efficiency of extraction. It was observed that any drug particles that were floating settled immediately when the vacuum was broken but this was not the case in the controls. However the quantitative data indicate that this was not due to quantitatively greater imbibition by the marc as a whole.

Experiment 3.—Experiments were next carried out using a higher vacuum and a larger quantity of drug. The vacuum was applied to the dry drug, the menstruum added and the vacuum broken and reapplied to the mixture several times.

Four hundred grams of moderately coarsely powdered belladonna root were placed in a five-liter, round-bottomed flask. Using a two-holed rubber stopper, one opening was connected to the vacuum pump by means of a glass tube and a separatory funnel was placed in the other opening. The flask was then evacuated for thirty minutes to a pressure of 14 mm. of mercury. Sixteen hundred grams of menstruum were gradually added through the separatory funnel with occasional shaking, taking fifteen minutes for the addition of the menstruum. The vacuum gage then showed a pressure of 50 mm. of mercury. Air was then allowed to enter the flask and the contents were thoroughly shaken. The mixture was allowed to stand at atmospheric pressure for ten minutes with occasional shaking. A partial vacuum (pressure of 65 mm. of mercury) was then applied for ten minutes with occasional shaking after which the mixture was allowed to stand at atmospheric pressure for ten minutes. Partial evacuation for ten minutes was repeated. The mixture was filtered and the macerate weighed and assayed. The damp marc was weighed.

In the above procedure evacuation was not carried below a pressure of 65 mm. of mercury after the menstruum had been added because boiling of the menstruum occurred at pressures of 42 to 47 mm. of mercury.

The experiment was repeated and in each case a control without vacuum was carried out at the same time. It was observed that the vacuum flask was cooler than the control flask, due to the cooling effect brought about by evaporation of some of the solvent, under reduced pressure. Since this temperature difference might affect the results to some extent further experiments were carried out in which the temperature was equalized.

The experiment and control were carried out in duplicate. Four hundred grams of moderately coarsely powdered belladonna root were placed in a five-liter, round-bottomed flask which was then evacuated to a pressure of 9 mm. of mercury for thirty minutes. The temperature within the flask was then 17° C. compared with 21° C. in the control. Sixteen hundred grams of menstruum were added through the separatory funnel in fifteen minutes with occasional shaking. The temperature in the vacuum flask was then 18° C. compared with 21° C. in the control. Air was admitted and the vacuum flask was then placed in a water-bath at 30° C. until the temperature of the contents was brought to 21° C. as in the control, this temperature being reached in eleven minutes. The contents of both flasks were thoroughly shaken. At this point the contents were filtered without reapplying vacuum as was done in the preceding experiment.

TABLE III.—EFFECT OF REDUCED PRESSURE ON EXTRACTION AND IMBIBITION OF 400-GM. PORTIONS OF POWDERED BELLADONNA ROOT.

	Weight in Gm. of					
	Liquid Imbibed in Marc.	Dry Marc.	Macerate.	Loss of Menstruum.	Total Extractive.	Total Alkaloids.
A. Vacuum Applied Both before and after Adding Menstruum.						
	696.4	307.1	968	28.5	37.4	1.09
	700.5	305.0	965	29.5	39.5	1.17
Av.	698.5	306.1	967	29.0	38.5	1.13

B. Control for Part A (No Vacuum Used).						
	705.6	306.4	978	10.0	38.1	1.14
	695.2	306.3	992	6.5	38.2	1.16
Av.	700.4	306.4	985	8.3	38.2	1.15
C. Vacuum Applied before Adding Menstruum (Temperature Equalized).						
	668.5	306.1	1007	18.5	38.4	1.14
	695.4	309.6	974	21.0	35.0	0.95
Av.	682.0	307.9	991	19.8	36.7	1.05
D. Control for Part C (No Vacuum Used).						
	648.0	303.0	1043	6.0	41.5	1.15
	670.6	303.4	1020	6.0	41.1	1.14
Av.	659.3	303.2	1032	6.0	41.3	1.15

The results in Table III indicate that vacuum is of no benefit in the extraction of belladonna root by maceration.

DISCUSSION OF RESULTS.

Effect of Vacuum in Imbibition.—Various writers have presumed that vacuum maceration increases the penetration of menstruum into the drug. It has been said that the application of vacuum withdraws all air from the finely divided drug so that the menstruum has free entry and that when the vacuum is broken the pressure of the atmosphere forces the menstruum into the drug. These views have been advocated by Duffield (27), Fairthorne (28), Peck (41) and Kessler (42). On the other hand Breddin (43) has stated that the removal of air has no value; if such a process does actually have the effect of introducing more solvent into the drug cells, such additional menstruum would have a diluting effect, in his opinion. The statements of the various authors apparently have been based on opinions rather than on facts, as no quantitative determinations have previously been published.

Hence, experiments were carried out in the present study in order to determine in a quantitative manner the effect of vacuum on the proportion of menstruum imbibed by the powdered drug as well as to determine the effect of vacuum on the efficiency of extraction. The results indicate that moderately coarsely powdered belladonna root does not imbibe any more menstruum in vacuum maceration than in ordinary maceration.

Effect of Vacuum on Efficiency of Extraction.—Peck (41) concluded from his experiments that vacuum maceration increased the total extractive in the preparation of tinctures by the maceration process. Such conclusions are not in accord with the results of the present study in which it is shown that in macerating belladonna root with an excess of liquid the use of vacuum does not increase the extraction of alkaloids or total extractive.

There are two possible explanations for the apparent conflict between our conclusions on belladonna root and those of Peck on vanilla beans and compound tincture of gentian. In the first place a close examination of Peck's data indicates that the differences between the vacuum experiments and controls were small and were not always in favor of vacuum, hence the differences may be within the experimental error. In the second place, since Peck withdrew equal samples from time to time for analysis it is possible that his results were not corrected for loss of solvent. If correction is not made for loss of solvent, it is to be expected that the product ob-

tained under reduced pressure will contain a slightly higher concentration of total solids than the control. This is due to the fact that when vacuum is applied to a liquid, the liquid evaporates more rapidly and more of the liquid is lost, thus concentrating the extracted matter in the macerate. This error was eliminated in our experiments by weighing each macerate and calculating the extracted material for the entire macerate. Hence, our experiments show conclusively that vacuum does not increase the efficiency of extraction of belladonna root in a maceration process.

SUMMARY.

A review of the literature indicates that pressure and vacuum have been used in various ways in drug extraction for more than one hundred and twenty years.

Various writers have presumed that vacuum maceration increases the penetration of menstruum into the drug. Quantitative determinations carried out in the present study indicate that moderately coarsely powdered belladonna root does not imbibe any more menstruum in vacuum maceration than in ordinary maceration.

Experiments show conclusively that the application of vacuum in different ways does not increase the efficiency of extraction of belladonna root in a maceration process.

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DRUG EXTRACTION. XX. THE EFFECT OF VACUUM ON THE PERCOLATION OF BELLADONNA ROOT.*¹

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In a previous study (1) it was found that the application of vacuum did not affect the efficiency of extraction of belladonna root in a maceration process.

Further research has been carried out to determine the efficiency of ordinary percolation of belladonna root as compared with a process of vacuum maceration in a percolator followed by ordinary percolation.

EXPERIMENTAL PART.

The drug and menstruum used and the methods of analysis were the same as in a previous study (1). In all experiments reported in this paper the drug was introduced into the percolators in small portions with slight agitation to promote even distribution, and after all the drug had been thus introduced it was packed from the top, starting with light pressure which was gradually increased.

Experiment 1.—A 250-Gm. portion of moderately coarsely powdered belladonna root was packed in the dry state in a pyrex glass tube 64 cm. in length and 4 cm. in internal diameter. The tube containing the dry drug was evacuated to a pressure of 9 mm. of mercury for thirty minutes, by means of a vacuum pump connected to the receiving flask. The menstruum was then added under reduced pressure. When the menstruum reached the lower orifice of the percolator tube air was admitted and the mixture allowed to macerate for twenty-four hours at atmospheric pressure. Percolation was then carried out at atmospheric pressure, two successive 125-cc. portions of percolate being collected. The experiment was carried out in duplicate and at the same time two controls were conducted, following the same procedure except that vacuum was not used.

In each case the volume of the packed drug was 505 cc. and the length of the drug column was between 37 and 38 cm. In the vacuum experiments the liquid reached the lower orifice in about 210 minutes while in the controls a time of about 1415 minutes was required. The rate of flow was 0.6 to 0.7 cc. per minute in the vacuum experiments and about 0.3 cc. per minute in the controls.

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