be lost during the ashing or the destruction of the organic material by other means, we urge that the question be studied, especially as to how best to destroy the organic material, if it is desired that organic medicinals be as free from metallic contamination as the inorganic medicinals, and that the prior destruction of organic materials be made a part of the U. S. P. heavy metals test. Further we suggest that, if it should be found difficult to prevent the presence of some traces of metallic impurities in organic medicinals, it would be advisable to further amplify the U. S. P. tests to show the identity of the metal present and indicate the amount permissible. A toxic metal such as mercury or lead should be strictly limited in concentration, while more leniency might be shown to less toxic or harmless ones.

DRUG EXTRACTION. XXII. THE EXTRACTION OF PODOPHYLLUM.*,[1]

BY WILLIAM J. HUSA[2] AND D. W. LEE.[3]

In a previous study (1) it was found that alcohol was preferable to alcohol-water mixture (9:1) for the preparation of resin of podophyllum. Further research has been carried out to determine the relative efficiency of various methods of extraction as applied to podophyllum.

EXPERIMENTAL PART.

Materials Used.—Podophyllum, U. S. P. in fine powder (No. 60) obtained from two different sources was used. Drug A contained 7.35% resin and 7.24% moisture. Drug B contained 5.69% resin and 7.66% moisture. Alcohol was used as the menstruum.

Analytical Methods.—Resin determinations were made by the U. S. P. XI method. Moisture was determined by the U. S. P. XI method for drugs containing no constituents volatile at 100° C. To determine total extractive, 10 cc. of liquid were evaporated to apparent dryness on a water-bath, the residue treated with 1 cc. of absolute alcohol and again evaporated to dryness on a water-bath; the residue was then heated in an oven at 105° C. until the loss in weight between two successive weighings did not exceed 5 mg. Due to the hygroscopic nature of the resin, the balances were equipped with balance desiccators.

Experiment 1. Maceration with an Excess of Alcohol.—An experiment was conducted to determine the time necessary for the establishment of equilibrium in the maceration of podophyllum with an excess of alcohol, using the centrifuge method developed by Husa and Magid (2).

Into each tared, wide-mouth bottle of about 250-cc. capacity were placed 10 Gm. of drug and 90 Gm. of alcohol. The bottles were then placed in a cabinet at 22–25° C. for 15 minutes, during which time the bottles were gently shaken every 5 minutes. The bottles were then centrifuged at about 1000 r. p. m. for ten minutes and allowed to stand 5 minutes. The clear supernatant liquid was decanted into tared, glass-stoppered bottles and weighed; the bottles containing the wet marc were also weighed. The macerate was adjusted to 20° C. before withdrawing samples for assay. The same technique was used for 12-hour, 24-hour and 36-hour maceration periods except that the bottles were shaken at convenient irregular intervals.

Calculations were made as follows: (Weight of drug) minus (weight of moisture in drug) minus (weight of total extractive in macerate) = (weight of dry marc). (Weight of wet marc) minus (weight of dry marc) = (weight of liquid imbibed by the marc). The loss of menstruum

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[1] This paper is based on a thesis presented to the Graduate Council of the University of Florida by D. W. Lee, in partial fulfilment of the requirements for the degree of Master of Science in Pharmacy.

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during the process was determined by comparing the total weight of the materials used with the combined weight of the macerate and wet marc.

The results in Table I are averages of duplicate assays of single macerations; the results were duplicated in another experiment which is not reported in detail.

TABLE I.—MACERATION WITH AN EXCESS OF ALCOHOL.

Period of Maceration.	Weight in Gm. of Liquid Imbibed in Marc.	Dry Marc.	Macerate.	Total Extractive.	Resin.
Drug A					
15 minutes	8.64	9.09	82.07	0.191	0.187
12 hours	8.65	8.92	82.41	0.358	0.312
24 hours	9.60	8.65	81.52	0.628	0.536
36 hours	9.76	8.65	81.57	0.628	0.548
Drug B					
15 minutes	7.60	9.06	83.21	0.173	0.173
12 hours	8.76	8.76	82.38	0.472	0.404
24 hours	7.84	8.69	83.25	0.549	0.431
36 hours	8.40	8.68	82.87	0.552	0.433

Assuming that the liquid imbibed in the marc contained the same concentration of dissolved resin as the macerate, calculations were made to determine the distribution of the resin at different stages of maceration. The results are given in Table II.

TABLE II.—DISTRIBUTION OF RESIN IN MACERATION WITH AN EXCESS OF ALCOHOL.

Period of Maceration.	Percentage of Resin. In Liquid Imbibed in Marc.	In Macerate.	Left Undissolved in Marc.
Drug A			
15 minutes	2.7	25.3	72.0
12 hours	4.3	42.4	53.3
24 hours	9.0	72.9	18.1
36 hours	9.0	74.6	16.4
Drug B			
15 minutes	2.9	30.5	66.6
12 hours	7.6	71.0	21.4
24 hours	7.0	75.4	17.6
36 hours	7.1	76.1	16.8

Tests were carried out on Drug B to determine whether a higher proportion of resin would be extracted by using a longer maceration period and increasing the quantity of menstruum. The technique was the same as in the preceding experiments except that 90 Gm. of menstruum were used in two bottles and 135 Gm. of menstruum in two others; in all cases the time of maceration was one week. The results are given in Table III. The calculated distribution of resin is shown in Table IV.

TABLE III.—MACERATION WITH AN EXCESS OF ALCOHOL FOR ONE WEEK USING DRUG B.

	Weight in Gm. of Liquid Imbibed in Marc.	Dry Marc.	Macerate.	Total Extractive.	Resin.
		Using 90 Gm. of Menstruum.			
	8.05	8.75	82.95	0.485	0.433
	8.15	8.75	82.81	0.487	0.429
Average	8.10	8.75	82.88	0.486	0.431
		Using 135 Gm. of Menstruum.			
	8.42	8.62	127.6	0.619	0.495
	8.20	8.63	127.9	0.609	0.463
Average	8.31	8.62	127.8	0.614	0.479

TABLE IV.—DISTRIBUTION OF RESIN AFTER MACERATION FOR ONE WEEK USING DRUG B.

	Percentage of Resin.		
	In Liquid Imbibed in Marc.	In Macerate.	Left Undissolved in Marc.
	Using 90 Gm. of Menstruum.		
	7.4	76.1	16.5
	7.4	75.4	17.2
Average	7.4	75.8	16.8
	Using 135 Gm. of Menstruum.		
	5.7	87.0	7.3
	5.2	81.4	13.4
Average	5.5	84.2	10.3

Experiment 2. Percolation.—A 250-gram portion of drug was packed in the dry state in a pyrex glass tube 64 cm. in length and 4 cm. in internal diameter. The drug was introduced into the percolator tube in small portions with slight agitation to promote even distribution and after all the drug had thus been introduced it was packed from the top, starting with light pressure which was gradually increased. The menstruum was added and when the liquid reached the lower orifice the flow was stopped for a maceration period of 12 hours. Percolation was then allowed to proceed; in each case the following successive portions of percolate were collected: 125 cc., 125 cc., 250 cc., 250 cc., 500 cc. The rate of flow was from 0.02 to 0.08 cc. per minute.

To determine the effect of tightness of packing, some tubes were packed lightly and some firmly. With light packing the volume of packed drug was 390 to 395 cc. and the length of the drug column was 290 to 295 mm.; with firm packing the volume of packed drug was 350 to 355 cc. and the length of the drug column was 260 to 265 mm. The time required for the liquid to reach the lower orifice was 455 to 480 minutes for the lightly packed drug and 968 to 1080 minutes for the firmly packed drug.

TABLE V.—PERCOLATION OF PODOPHYLLUM.

	Resin Extracted in Gm.					
	Drug A.			Drug B.		
Fraction.	Light Packing.	Firm Packing.	Firm Packing.	Light Packing.	Firm Packing.	Firm Packing.
I (125 cc.)	9.09	14.71	14.45	10.04	11.58	11.91
II (125 cc.)	5.41	1.87	1.32	2.60	1.13	0.93
III (250 cc.)	1.40	0.84	0.73	0.52	0.43	0.49
IV (250 cc.)	0.50	0.31	0.52	0.20	0.21	0.21
V (500 cc.)	0.44	0.49	0.38	0.22	0.22	0.28
Total (1250 cc.)	16.84	18.22	17.40	13.58	13.57	13.82
	Percentage of Resin Extracted.					
I (125 cc.)	49.5	80.0	78.6	70.1	81.4	83.7
II (125 cc.)	29.4	10.1	7.2	18.3	8.0	6.6
III (250 cc.)	7.6	4.5	3.9	3.7	3.0	3.5
IV (250 cc.)	2.7	1.7	2.8	1.4	1.5	1.5
V (500 cc.)	2.4	2.7	2.0	1.5	1.5	2.0
Total (1250 cc.)	91.6	99.0	94.5	95.0	95.4	97.3
	Total Extractive in Gm.					
I (125 cc.)	10.84	17.33	17.17	11.42	13.18	13.55
II (125 cc.)	6.47	2.82	2.07	3.41	1.91	1.62
III (250 cc.)	2.49	1.59	1.45	1.64	1.41	1.31
IV (250 cc.)	1.06	1.03	1.07	0.92	0.96	0.80
V (500 cc.)	1.65	1.74	1.47	1.13	1.00	1.06
Total (1250 cc.)	22.51	24.51	23.23	18.52	18.46	18.34

Experiment 3. Forced Percolation through a Long Column of Drug.—Using Drug B, tests were conducted to determine the efficiency of extraction of podophyllum by the method of forced percolation through a long column of drug. The apparatus previously described by Husa and Huyck (3) was employed. A long column of drug was obtained by using pyrex glass pipes joined together by means of U-shaped fittings made of pyrex glass pipe. The dimensions of the tubes were as follows: length, 91 cm.; internal diameter, 2.5 cm.; thickness of wall, 5 mm. Three percolations were conducted in order to have variations in the number of tubes and tightness of packing. The menstruum was forced through the drug with air pressure which was gradually increased from a slight pressure to a maximum of 30 lbs. per square inch. In experiments 3-*A* and 3-*B* as much drug as possible was placed loosely in each tube and then packed from the top; in experiment 3-*C* the drug was introduced in 20-Gm. portions with moderate packing after each addition. Before packing, the drug was moistened with 25 cc. of menstruum per 100 Gm. of drug.

During the extraction several breaks in the drug column were observed in 3-*A*, such as might be due to contraction of the drug on removal of extractive or to settling of the drug. In 3-*B* the breaks were numerous and extensive because the drug had been packed very lightly. In 3-*C* no breaks occurred in the drug column during percolation.

TABLE VI.—FORCED PERCOLATION THROUGH LONG COLUMN OF DRUG.

Experimental Data.

Experiment.	Number of Tubes.	Gm. of Drug Used.	Volume of Packed Drug in Cc.	Time in Hrs. for Menstruum to Reach Orifice.	Time of Collections of Fractions in Hours. Fraction I.	Fraction II.
3-*A*	8	3275	5640	440	316	..
3-*B*	6	1861	4230	130	34	33
3-*C*	6	2270	4230	136	82	74

	Fraction I.			Fraction II.		
	Volume in Cc.	Gm. Resin.	Gm. Total Extractive.	Volume in Cc.	Gm. Resin.	Gm. Total Extractive.
3-*A*	1637.5	166.5	193.6	..	..	..
3-*B*	930.5	89.5	102.6	930.5	6.5	21.1
3-*C*	1135.0	123.6	144.1	1135.0	4.2	12.5

Percentage of Resin Extracted.

Fraction.	Exp. 3-*A*.	Exp. 3-*B*.	Exp. 3-*C*.
I (0.5 cc. per 1 Gm. drug)	89.3	84.5	95.7
II (0.5 cc. per 1 Gm. drug)	..	6.1	3.3
Total (1 cc. per 1 Gm. drug)		90.6	99.0

DISCUSSION OF RESULTS.

Maceration with an Excess of Alcohol.—The data in Tables II and IV indicate that equilibrium is attained in 36 hours in macerating 10 Gm. of podophyllum with 90 Gm. of alcohol. It appears that 90 Gm. of alcohol are insufficient for complete extraction of 10 Gm. of podophyllum at room temperature; even with 135 Gm. of alcohol about ten per cent of the resin remained undissolved. In this respect podophyllum differs from belladonna root, since Husa and Magid (2) using belladonna root showed that as much of the alkaloid was extracted in 15 minutes as in 24 hours with Nos. 40, 60 and 80 powders, but not with No. 20 powder, which gave a maximum yield of alkaloids after one hour. In the case of jalap, Husa and Fehder (4) found that equilibrium in the extraction of resin was reached within 15 minutes.

Table I indicates that the material extracted from podophyllum during the first 15 minutes of maceration was practically all resin. It was also found that the

resin was not extracted at the same rate from two different samples of drug. The drug containing the lower percentage of resin was extracted more rapidly than the drug containing the higher percentage of resin. Possibly the percentage of resin is higher in older plants whose tissues may become less permeable with increasing age.

Extraction by Percolation.—The results in Table V indicate that when podophyllum was packed firmly a greater proportion of the resin was extracted in the first fraction of percolate than was the case with light packing. However, the quantity of resin extracted in 1250 cc. of percolate from 250 Gm. of drug was almost the same for both methods of packing. Using pyrex glass tubes 64 cm. long and 4 cm. in internal diameter, it was found that the first 125-cc. portion of percolate from 250 Gm. of drug contained most of the resin. The first 250 cc. of percolate from 250 Gm. of drug contained about 85 to 90 per cent of the total resin of the drug.

Forced Percolation through a Long Column of Drug.—Using the method of forced percolation through a long column of drug it was found that as much as 99 per cent of the resin could be extracted in the first 1000 cc. of percolate from 1000 Gm. of drug, compared with 85 to 90 per cent with a shorter drug column. Best results were obtained when the drug was packed in small successive portions with moderate firmness in such a manner that no breaks in the drug column occurred during percolation. Table VI shows that best results were obtained in 3-*C*, in which no breaks occurred in the drug column, next best results were observed in 3-*A* which showed some breaks, and poorest results were obtained in 3-*B*, which showed numerous breaks in the drug column. When breaks occur in the drug column the open spaces become partly filled with liquid and at these points there would doubtless be more diffusion and less efficient displacement of the more saturated liquid by less saturated liquid.

SUMMARY.

Podophyllum was extracted by various methods. In the process of maceration of podophyllum with an excess of alcohol, equilibrium was reached in 36 hours. By percolation in cylindrical pyrex glass tubes, the first 250 cc. of percolate from 250 Gm. of drug contained 85 to 90 per cent of the total resin of the drug. In a method of forcing the menstruum through a long column of drug by means of air pressure, as much as 99 per cent of the resin was obtained in the first 1000 cc. of percolate from 1000 Gm. of drug.

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Establishment of a new laboratory for the study of filterable virus diseases, in the treatment and prevention of which science is believed to be at the threshold of an important advance, is announced by the Squibb Biological Laboratories.

Dr. Raymond C. Parker, biologist of the Rockefeller Institute for Medical Research, and for many years an associate of Dr. Alexis Carrel, has been appointed to head the laboratory, which will operate as a unit of the Biological Division of E. R. Squibb and Sons at New Brunswick, N. J. The new building is a continuation of a program of expansion which began in the fall of 1938 with the dedication to pure science of the $750,000 laboratory of the Squibb Institute for Medical Research.