most effectively demonstrated by the fact that after the preparation of fields for abdominal operations no so-called iodine blisters are found, even on the marginal areas where the excess solution from the field collects and usually concentrates.

Effectiveness.—The concentrations mentioned above have been chosen on the basis of their clinical effectiveness, and on further evidence of their bactericidal effectiveness, as judged by accepted means of test. Bacteriological data on the solution in question will be presented with data on other iodine solutions in a later paper by Gershenfeld and Miller. The comparative phenol coefficients of various iodine solutions demonstrate the importance of considering dosage in terms of surface concentration. The aqueous solution in question has a phenol coefficient that is about 15 per cent greater than an iodine solution of like strength made up with alcohol. It is unlikely that this effect is due to the favorable action that alcohol has exhibited on the growth of the bacteria or that it has chemically inhibited the effect of the iodine. When the surface behavior of the iodine in the two solutions is considered, it is more logical to suppose that, even though the alcoholic solution is approaching an aqueous solution at the dilution where the final determination of the phenol coefficient is made, the difference in surface concentration is still appreciable. The importance of surface effects as influenced by the solvent would be much more marked in solutions used without dilution of the solvent. The magnitude of these differences will remain a matter of conjecture pending the development of tests for the bacteriological evaluation of antiseptic solutions under the conditions of use.

<div align="center">ACKNOWLEDGMENTS.</div>

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PITTSBURGH, PA.

ISOLATION AND STUDY OF THE SAPONIN CONTENT OF THE JUICE AND LEAF OF THE AGAVE PLANT, MAGUEY, MANSO FINO.*

BY HERMAN D. JONES,[1] WITH THE ASSISTANCE OF G. NELSON FURBECK AND ROUL COLORADO.

The saponin occurs in two groups of agaves which are known as the Amoles and Magueys (1). The leaves and roots of the Amoles are sold in the markets as a substitute for soap because of the ability of the saponin content to lather freely in water. The Magueys are known to the Mexicans as Pita Magueys, Mescal Magueys and Pulque Magueys. The latter are grown in the region of Ometusco in the State of

Mexico. They are classified as Maguey, Manso Fino (Agave Atrovirens, Karw), and are the ones from which the saponin reported in this communication was obtained.

Saponins are non-nitrogenous glucosides and are abundant in the vegetable kingdom. Aqueous solutions produce abundant foaming upon agitation and are capable of holding in suspension bodies insoluble in water. Aqueous solutions also dissolve the red corpuscles of the blood. The dry powder produces sneezing and irritation of the skin; with sulphuric acid they give a red to violet color.

Almost all the saponins are colorless, amorphous compounds, easily soluble in water, insoluble in absolute alcohol, but as the water content of the alcohol is increased they become more soluble until appreciable quantities are soluble in 70 to 80% alcohol, especially hot 70% alcohol. They are also soluble in methyl and amyl alcohol, and to some degree, in phenol. They are insoluble in such solvents, as ether, petroleum ether, benzol and chloroform.

The saponin in these studies was obtained by three methods. The first one used was that of L. Rosenthaler, in which the saponin was precipitated by lead acetate from the fresh aguamiel and also from the juice by pressing from the fresh leaves. The precipitate was washed and broken up with excess dilute sulphuric acid. The lead sulphate formed was removed by filtering and the last traces of lead acetate were removed from the filtrate by passing in hydrogen sulphide, and by filtering to remove the lead sulphide. The volume was reduced by evaporation over water-bath to approximately the original volume or to a syrupy consistency. This residue was washed thoroughly with hot alcohol, and the alcohol solution separated by decantation. The saponin was then precipitated from the alcoholic solution by the addition of excess ether.

As this was a long process and very small quantities of the saponin were obtained, an attempt was made to obtain the substance in larger quantities. Knowing the solubility of the saponin in 70% alcohol and that large quantities were present in the dry leaves of the Maguey, Manso Fino, it was decided to try to obtain it from this source.

The dry leaves were powdered and the powder passed through a 40-mesh sieve to separate it from the fibre. This powder was then extracted thoroughly with hot, distilled water. Large quantities of the saponin and also of the gums were obtained. The solution was cooled and the gums removed by fractional precipitation with 96% alcohol, each time the excess alcohol being removed by distillation over water-bath and under vacuum, and the volume of the solution reduced. After removing all the gums, the residue, which was largely an alcoholic solution, was treated with excess ether, which caused the precipitation of large quantities of saponin. This method has the disadvantage that large quantities of the gums, very soluble in hot water, are obtained and much time is required to remove them before precipitation of the saponin.

The third method used was as follows: The dry powder, free from the fibre, was extracted for 24 hours with sulphuric ether to remove all oily and resinous substances.[1] The powder was then removed from the extractor and the last traces of the ether removed by exposing it to the air over large areas. After all the ether had evaporated, the powder was replaced in the extractor and extracted for eight hours

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[1] A study of these substances is now being made.

with 70% alcohol. Small quantities of the gums present, being soluble in water, came down with the saponin. The larger quantities, however, remained in the powder, being insoluble in alcohol. After the extraction was completed, the traces of gum were removed by adding 96% alcohol in excess. The solution was filtered to remove the precipitated gum. The excess alcohol was then removed by distillation over water-bath and under vacuum. The residue was cooled and excess ether added. Large quantities of the substance were obtained by this procedure.

In each method used, the precipitated saponin required 24–36 hours to settle to the bottom of the container. The excess ether and alcohol were then poured off. The saponin, as obtained after precipitation, carries some ether and alcohol, which tend to hold it in the form of a heavy solution. This is placed in the oven at 35°–40° and allowed to remain until perfectly dry. The residue is then broken up and powdered, the resulting substance being a light, tan-colored amorphous powder.

CHEMICAL AND PHYSICAL STUDY.

Light tan, amorphous powder. Absorbs moisture on standing.

Melting point—decomposes, turning dark brown.

With sulphuric acid, turns dark red.

With sulphuric acid and potassium permanganate, decolorizes the permanganate solution completely, also from a solution to which sulphuric acid has been previously added and the red color fully developed on addition of permanganate, the color completely disappears. p_H-5, Hellige Comparator, methyl red indicator.

Solubility—completely soluble in water; slightly soluble in 96% alcohol, easily soluble in hot 70% alcohol; insoluble in ether and petroleum ether.

Has rather bitter, acrid, unpleasant taste, producing a free flow of saliva.

Produces irritation when rubbed on the skin and slightly attacks the mucous membranes of the nostrils.

Analysis for Elements.—Nitrogen, sulphur, phosphorus and halogens negative; calcium, carbon, hydrogen present.

Calcium is found to be present in quantity of 0.41%.

Combustion Analysis.—On combustion, analysis showed carbon 48.44%; hydrogen, 7.2%; oxygen calculated 43.95%, giving an empirical formula of C_2H_3O. On calculating for the molecular weight by the boiling point method, it was found to be approximately 690. This gives a molecular formula of $C_{32.4}H_{48.6}O_{16.2}$. When making this to whole numbers to the nearest decimal, it becomes $C_{32}H_{49}O_{16}$. When comparing this with the formula as worked out by Noyes, it is found that there is a difference of H_3O, as Noyes gives the formula $C_{32}H_{52}O_{17}$. This error is probably due to the presence of the calcium, which has a tendency to hold back carbon and oxygen. This analysis was checked three different times, obtaining the same results each time. On referring to the literature, it is found that there are two general formulas given for saponins, $C_nH_{2n-10}O_{18}$ (Flückiger) and $C_nH_{2n-8}O_{10}$ (Kobert). It is obvious that the formula as determined for this saponin conforms more nearly to Flückiger's than Kobert's.

The method used for making the combustion analysis is given below. The dry saponins are very hydroscopic and care was taken to prevent the absorption of any water in making the analysis. The saponin was placed in a desiccator over sulphuric acid for 48 hours before making the analysis, the container being a ground

glass, stoppered weighing pan. On commencing the analysis, the desiccator was opened quickly and the weighing pan immediately closed. It was then placed on the balance and the weight recorded. A small quantity was removed from the weighing pan and transferred to a boat previously prepared, containing a mixture of lead chromate and potassium bichromate. The saponin was immediately covered with another layer of the chromate mixture and placed in the combustion tube. The entire process of transferring and placing in the tube required not more than 30 seconds. The combustion was carried on from this point in the usual manner.

HEMOLYTIC ACTION.

The hemolytic action of the saponin was tested according to the method given in the German Pharmacopœia. Fresh blood was taken from a rabbit and defibrinized. One cc. of the defibrinized blood was diluted with 49 cc. of sterile, physiologic salt solution; 5 cc. of this blood solution was placed in each of seven small test-tubes of equal size. The first and seventh were used as controls, to each of which was added 5 cc. of physiologic salt solution. To the other five tubes of the series was added 1 cc., 2 cc., 3 cc., 4 cc., 5 cc., respectively, of the saponin solution and 4 cc., 3 cc., 2 cc., 1 cc. and 0 cc., respectively, of the physiologic salt solution. The saponin solution was made so that 1 cc. contained 10 mg. of the saponin. In the control tubes, there was 10 cc. of blood diluted to 1% and the other five tubes contained 10, 20, 30, 40 and 50 mg. of the saponin.

Results.—Slight hemolysis was noted immediately. The first four tubes showed complete hemolysis in 2 to 3 hours, print being perfectly legible through the tubes in that time, whereas the controls showed none, even at the end of 24 hours.

HEMOLYTIC ACTION OF THE SAPONIN IN THE WHITE RAT.

The saponin was given orally to five white rats, weighing 153 Gm., 120 Gm., 152 Gm., 150 Gm. and 115 Gm., respectively. The first animal received 20 mg., the second 30 mg., the third 50 mg., the fourth 63 mg. and the fifth 84.5 mg., after an original blood count had been made on each animal. At the end of 30 minutes after the administration of the saponin a second blood count was made on each animal, followed by additional counts at intervals of 15 minutes.

Results:

	Animal No. 1.	Animal No. 2.	Animal No. 3.
1st count Before injection	8,760,000	8,448,000	8,576,000
2nd count 30 min. after injection	7,456,000	6,800,000	7,578,000
3rd count 45 min. after injection	7,466,000	5,800,000	7,220,000
4th count 1 hour after injection	7,376,000	5,420,000	6,880,000

In animals No. 4 and No. 5 counts were made over a period of several days in order to establish their normal blood count before actually beginning the experiment.

Results:

NORMAL COUNTS.

Date.	Animal No. 4.	Animal No. 5.
May 7th	8,026,665	8,340,000
May 9th	8,448,000	8,224,000
May 10th	8,160,000	8,144,000
May 13th	8,120,000	8,040,000

Results:

	Animal No. 4.	Animal No. 5.
Normal count	7,649,000	8,395,000
On day of injection		
2nd count	4,781,000	4,675,300
30 min. after injection		
3rd count	5,354,600	5,061,600
45 min. after injection		
4th count	5,927,400	5,720,000
1 hour after injection		

Results:

No change in the animals was noted by the observers. The saponin given did not produce vomiting and did not seem to affect the nervous system and did not cause diarrhea. But it is seen from the above results that the red blood cell count was reduced.

HEMOLYTIC ACTION OF SAPONIN WHEN ADMINISTERED SUBCUTANEOUSLY.

White rats, two in number, were injected subcutaneously with 40 mg. each of the saponin.

Results:

	Animal No. 6.	Animal No. 7.
1st count	7,968,000	8,000,000
Before injection		
2nd count after injection	7,080,000	5,808,000
3rd count, 45 min.	4,880,000	5,800,000
4th count, 1 hour	5,280,000	7,784,000

Systemic Action.—The nervous system of animals in these two experiments was greatly affected. They were unable to control the leg muscles or walk, but hopped. At the end of one hour the hind legs of animal No. 6 were completely paralyzed and it was in a semi-comatose state, insensible to touch, with heart action greatly accelerated. The flow of blood in extremities had practically ceased. After approximately two and a half hours it began to be slightly sensitive to touch. After three hours it showed slight signs of activity and better control of limbs. After twelve hours it was still inactive but control of leg muscles was slightly better. The general condition was much improved over that of first two and a half hours. However, this condition did not persist, the animal taking no food and having no excretions. Thirty-six hours later it developed slight convulsions occurring at intervals of 15 to 20 minutes. Heart action had greatly decreased. The entire body had become cold, and weakness increased until the animal, unable to stand, lay on its side in the cage in a paralytic condition. Forty-four hours after the injection the animal was in a comatose state and died during the night.

Animal No. 7 showed greater resistance. At the end of 15 minutes there was little sign of nervous disturbance but at the end of 45 minutes, the hind legs were paralyzed, extremities of the body were insensible to touch and the blood flow

ceased. Twenty-four hours later the animal seemed to have recovered from the paralytic effect and began to eat, but had developed diarrhea. This condition lasted for six days. On the last day the animal was active and playing in its cage. At about ten-thirty he was noticed to be lying on his side completely paralyzed. This condition lasted about eight hours, until death occurred.

HEMOLYTIC ACTION OF THE SAPONIN WHEN INJECTED INTRAVENOUSLY IN A RABBIT.

A rabbit weighing 2018 Gm. was injected in the marginal ear vein with 80 mg. of saponin. This animal had been under observation for five weeks.

Results:		
	First blood count before injection	5,008,000
	Second—30 min. after injection	2,496,000
	Third, 1 hour 10 min. after injection	5,072,000

It is seen from these experiments that there was a large decrease in blood cell count in the first half hour, but also the cells seemed to be replaced in the blood stream within an hour and ten minutes.

Forty minutes after the injection of the saponin, the animal voided 50 cc. of bloody urine. Under microscopic examination no red corpuscles were found, but on testing for occult blood, it was found to be positive.

The nervous system was affected but not so severely as in experiments No. 6 and No. 7. This might have been due to the fact that the injection was not sufficiently strong for the larger animal, which might also have had greater resistance than the rats, even though the blood count was greatly decreased. There was twitching of the muscles of the entire body, with loss of control of head muscles. The animal was inactive for practically two hours, but three hours after the injection seemed to be recovering and was more or less active. There was no vomiting, no diarrhea and no more blood in the urine.

DISCUSSION.

In consulting the pharmacology of the saponins (2), (3), it is observed that these substances have a tendency to alter protoplasmic surface tension and have a special affinity for cholesterol.

The results as obtained might indicate that there was true hemolysis. Yet, when it is noted that the blood count began to increase after intervals of approximately 45 minutes to one hour, it is possible that the cells were not actually destroyed but were withdrawn temporarily from the blood stream by the liver, spleen, bone marrow and other organs of the body. After the body had built up a resistance to the toxic effect through the action of the lecithin and cholesterol, it is possible that the blood cells were again returned to the blood stream. However, it is seen that there was complete hemolysis *in vitro* and it is also seen that there was a true positive test for occult blood in the urine voided by the rabbit, indicating apparently that there was true hemolysis in this case. From these facts, one would be more inclined to believe that there was true hemolysis *in vivo*.

When the saponin was given orally in appreciable quantities, it did not produce such violent effects as when administered by injection. This might be due to the protective effect of the digestive juices or to the fact that the saponin was absorbed more slowly when taken orally than when placed in close proximity to the blood stream.

SUMMARY.

The observed effects of saponin herein reported are as follows: It has some hemolytic action, it does not produce nausea, but does affect the nervous system, and produces diarrhea when given by injection.   The leaf juice and fresh aguamiel contain very small quantities of the saponin.

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QUALITY OF JAPANESE PEPPERMINT OIL PRODUCED IN FLORIDA.[*][1]

BY. B. V. CHRISTENSEN AND LOVELL D. HINER.

It appears that the cultivation of Japanese peppermint (*Mentha arvensis var. piperescens*) was first begun on the island of Hondo, and for many years the industry was restricted to this area.   Late in the 19th Century, immigrants from Hondo to the more northern island of Hokkaido carried with them propagating stock from the mint fields of their native land.   With this stock they established the first mint plantations in Hokkaido, which by 1906, had so far surpassed Hondo in Japanese mint oil production that it produced about 92% of that produced in Japan.   For many years Japan has produced a large proportion of the world's supply of natural menthol and this product has been one of its most important commercial commodities.

Just how and when Japanese peppermint was introduced into the United States is not certain.   However, during the past ten or twelve years cultural experiments have been carried on in various parts of the United States.

A few years ago an experimental plot was established at Mont Verde, Florida, by an industrial concern, using large quantities of natural menthol, apparently for the purpose of determining the best location in the United States for the cultivation of this plant to furnish their needs.   This project was discontinued in 1926 on account of the low menthol content of the oil and apparently this firm decided that Florida was not the location in which to attempt cultivation on a large scale.   This, with one or two other attempts, seemed to convey the impression that the results obtained in the hotter sections of the United States, such as Florida, indicated that Japanese mint oil produced in these sections ran low in menthol.   It was also supposed that the menthol content gradually decreased from year to year and that the percentage of combined menthol was relatively high.

The Bureau of Plant Industry in Washington, D. C., became interested in the possibilities of this plant and in February 1927, it was proposed by A. F. Sievers of that Bureau that the College of Pharmacy, University of Florida, coöperate with the Bureau of Plant Industry in carrying out cultural tests in Florida with Japanese peppermint.   Rootstock was furnished by the Bureau and the first planting was

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* Produced in Medicinal Plant Garden, University of Florida.
[1] Scientific Section, A. Ph. A., Miami meeting, 1931.