

of the ingredients in N. F. Antiseptic Solution can be used.

5. When decreased amounts of the ingredients in N. F. Antiseptic Solution are used, providing chlorthymol is not less than 0.5 Gm. per liter, the antiseptic value is equal to that of the present official solution.

The following formula with working directions is offered for criticism. This solution when submitted to the test for antiseptic value, described for N. F. Antiseptic Solution, showed *Staphylococcus aureus* to be killed in 1/2 minute as shown by no growth on the subculture after incubation for 48 hours at 37.5° C.

Boric acid.....	25.00 Gm.
Chlorthymol.....	0.7 Gm.
Menthol.....	0.15 Gm.
Methyl salicylate.....	0.15 Gm.
Eucalyptol.....	0.15 Gm.
Oil of thyme.....	0.05 cc.
Alcohol.....	300.00 cc.
Distilled water <i>q. s.</i>	_____
To make.....	1000.00 cc.

Dissolve the boric acid in 650 cc. of warm distilled water and the other ingredients in the alcohol. Cool the boric acid solution, add it to the alcoholic solution with continual agitation; finally add sufficient distilled water to make 1000 cc. and mix well.

Assay of Sarsaparilla Preparations*

Preliminary Report

By B. Fantus, M.D., and H. A. Dyniewicz, Ph.C.

It is the consensus of modern medical opinion that the compound syrup of sarsaparilla is chiefly a flavoring vehicle (U. S. Dispensatory, page 1069, 21st Edition). This opinion was responsible for the deletion of the Fluidextract of Senna from the formula in the tenth revision as laxative effect might not be wanted in a mere flavoring agent. It may, indeed, be questioned whether the sarsaparilla might not be deleted from the formula, for the pleasant

flavoring qualities of this syrup are not due to the sarsaparilla at all but are due to the volatile oils contained in the preparation. Indeed, what is by common consent called the sarsaparilla flavor is really a "sassafras bouquet," and the title "Compound Syrup of Sassafras" would be preferable unless it can be shown that sarsaparilla has some pharmacologic and possibly therapeutic action.

The question is whether the presence of the sarsaparilla is of any advantage in the compound syrup.

Prof. Gathercoal's prescription survey (1) shows the usage of the Compound Syrup of Sarsaparilla has been 60 per 10,000 prescriptions for the last 30 years. With this considerable usage in mind, in spite of the unfavorable opinion of the pharmacologists as to any medicinal value of sarsaparilla, we undertook this study in an endeavor to find upon what effect, if any, the preparations may be assayed.

According to a report by F. W. Apt (2) sarsaparilla is dependent upon saponin for its activity, and the saponin increases absorption of the medicament that may be carried in a preparation of sarsaparilla. The U. S. Dispensatory (21st Edition, page 963) reads: "The sarsaparilla of commerce is apt to be nearly, if not quite inert, either from age or from having been obtained from inferior species. The only criterion of good sarsaparilla to be relied on is its taste. As it leaves a decided acrid impression in the mouth it is considered effective; otherwise it is inert."

There are in the literature numerous references on the increased absorption of various drugs by means of saponins as will be seen by consulting the bibliography (3 to 10). Hence the sarsaparilla assay should be for saponin.

According to R. Kobert (11) the activity of the saponins is best evaluated by the hemolysis of red blood cells in physiologic salt solution in a given time. This was substantiated by C. Sormani and J. Rühle (12) and K. Hering (13). A test based upon hemolysis was developed by these investigators and modified by W. Brandt (14). A table of 80 drugs giving

* From the Laboratory of Pharmacology and Therapeutics, University of Illinois College of Medicine; assisted by a grant from the AMERICAN PHARMACEUTICAL ASSOCIATION.

the hemolytic index for each drug was reported by A. Kuhn and G. Schaefer (15).

The results obtained in our experiments were mostly for comparative purposes. They were all done on the same day with the same blood sample so that all observations were made under similar conditions giving relative values of the different saponins, and also fluidextracts and syrups of sarsaparilla.

A direct comparison of the fluidextract and syrup cannot be made since the rate of hemolysis is less in sucrose than in normal saline solution itself (16). Comparative results of various syrups, however, were secured since the quantity of sucrose was constant.

EXPERIMENTAL

Method.—Freshly drawn rabbit's blood was defibrinated by shaking with glass beads. The corpuscles from 2 cc. of this blood were washed several times with physiological salt solution and finally suspended in 100 cc. of physiological salt solution.

To each test-tube containing 5 cc. of the defibrinated blood suspension an aqueous solution of the saponin (1 to 1000) was added in progressively increasing amounts: 0.1 cc., 0.2 cc., 0.3 cc., etc.; the mixture was made up to 10 cc. with physiologic salt solution. The saponins act directly on the cell according to E. Ponder (17) and belong to the simple hemolytic system. The criterion of complete lysis is the disappearance of all opacity or cloudiness in the tube. The tube containing the smallest amount of saponin hemolyzing completely in 5 minutes at room temperature, was taken as the reading and the "Hemolytic Index" calculated, *e. g.*,

0.1 cc. not complete in 5 minutes
0.2 cc. not complete in 5 minutes
0.3 cc. not complete in 5 minutes
0.4 cc. complete in 5 minutes
0.5 cc. complete in 5 minutes

The following results show the hemolytic index to be 0.0004 in 10 cc., or 1-25,000.

The hemolytic index is dependent upon the laking of blood due to the union of saponin with the cholesterin bodies. This we have found to vary for various specimens from 1:25,000 to 1:6250 (crude).

For the assay of fluidextracts, an aqueous dilution (2 to 100) of the fluidextract was used. The

TABLE I.—HEMOLYTIC INDEX (H. I.) OF VARIOUS SAPONINS

Saponin	Brand	Manufacturer's No.	In Dilution
No. 1	M-B	6080-3281	1-25,000
No. 2	M-B	6080-3281 (different container)	1-25,000
No. 3	M-B	6080-21848	1-14,300
No. 4	M-B	6081-32182	1- 9,100
No. 5	M-B	6081-32182 (different container)	1- 9,100
No. 6	M-B	6081-28264 (purified)	1-18,200
No. 7	F		1-14,300
No. 8	Crude		1- 6,250

This table shows that the same saponin, *i. e.*, having the same number as given it by a manufacturer gives the same assay even though taken from a different container.

TABLE II.—HEMOLYTIC INDEX OF VARIOUS FLUIDEXTRACTS

No. 1	(Reserved percolate, not subjected to heat)	1- 250
No. 2	(The combination of No. 1 and the pilular extract make the official fluidextract: only the extractive is subjected to heat)	1-1666
No. 3	(Jamaica sarsaparilla, wormy; fluidextract mucilagenous and murky)	1-1111
No. 4	(Honduras sarsaparilla)	1-1111
No. 5	(Mexican sarsaparilla)	1-1000
No. 6	(Commercial sample)	1- 666
No. 7	(Student's preparation—five years old)	1- 714
No. 8	(Student's preparation—one year old)	1- 333
No. 9	(Commercial sample)	1- 600
No. 10	(Commercial sample)	1- 294
No. 11	(Commercial sample)	1- 312
No. 12	(Laboratory, old sample)	1- 500

TABLE III.—HEMOLYTIC INDEX OF VARIOUS SYRUPS

No. 1	Syrup made from fluidextract No. 12 (months old)	1- 83
No. 2	Syrup made from fluidextract No. 12 (fresh)	1-100
No. 3	Syrup made from fluidextract No. 6	1-125
No. 4	Syrup from stockroom (old sample)	1- 62.5

hemolytic index of 12 different fluidextracts varied from 1:1666 to 1:300.

For the assay of syrups, an aqueous dilution (1 to 10) of the syrup was used. The hemolytic index of 4 different syrups varied from 1:62.5 to 1:125.

DISCUSSION

If saponin increases the absorption of drugs, then sarsaparilla may be more than a mere flavoring vehicle and should be assayed for its saponin. The hemolysis method seems to give satisfactory results. If accepted as an assay method for the official preparations, further experimentation will be necessary to standardize the assay. The results reported upon give relative values of the saponins, and also the fluidextracts and syrups of sarsaparilla. We have shown by means of the "hemolytic index" the variability of the saponins, fluidextracts and syrups of sarsaparilla upon the market to be so great that, if the medicinal effect of the preparations depended upon it, physicians might easily lose confidence in them. Further study of the possibility of using the hemolytic index for this purpose is indicated, and also a study as to whether the hemolytic index parallels the degree to which absorption of standard drugs are increased by the saponin.

BIBLIOGRAPHY

- (1) Gathercoal, E. N., "The Prescription Ingredient Survey," AMERICAN PHARMACEUTICAL ASSOCIATION (1933).
- (2) Apt, F. W., *Ber. deut. Pharm. Ges.*, 31, 155 (1921).
- (3) Kofler, L., and Fischer, R., *Arch. Exptl. Path. Pharmacol.*, 141, 105-115 (1929). Cholesterol absorption favored.
- (4) Hockstra, R. A., *Ibid.*, 163, 46-65 (1931). Digitalis absorption favored.
- (5) Lasch, F., *Biochem. Z.*, 169, 301-307 (1926). Calcium.
- (6) Lasch, F., and Brügel, S., *Ibid.*, 172, 422-427 (1926). Dextrose.
- (7) Lasch, F., and Brügel, S., *Arch. Exptl. Path. Pharmacol.*, 120, 144-155 (1927). Insulin.
- (8) Köfler, L., *Deut. med. Wochschr.*, page 488 (Sept. 16, 1932). Acetylsalicylic acid, camphor, methylene blue, hypophysis.
- (9) Annau, E., and Hergloz, J., *Arch. Exptl. Path. Pharmacol.*, 127, 93-100 (1927). Alkaloids, such as strychnine, curare, morphine, cocaine.
- (10) Fantus, B., and Dyniewicz, H. A. and J. M., *J. Pharm. & Exp. Therap.*, 55, 222-233 (1935). Acetanilide.

- (11) Kobert, R., *Pharm. Ztg.*, 57, 213-214 (1912).
- (12) Sormani, C., and Rühle, J., *Ztschr. f. Unters. d. Nahr. u. Genussn.*, 23, 11 (1912).
- (13) Hering, K., *Arch. Pharm.*, 268, 24-36 (1930).
- (14) Brandt, W., *Pharm. Ztg.*, 69, 204-205 (1924).
- (15) Kuhn, A., and Schaefer, G., *Ibid.*, 80, 257 (1925).
- (16) Handovsky, Hans, *Arch. ges. Physiol. (Pflüger's)*, 190, 173-176 (1921).
- (17) Ponder, Eric, "The Mammalian Red Cell and the Properties of Hemolytic Systems," Vol. 6 (1934).

A Monograph for Ringer's Solution as a Solvent*

By Norman Pinschmidt and John C. Krantz, Jr.†

The first work of note with physiological saline solutions was done by Sidney Ringer and his associates between the years 1882-1895. They advocated the use of the chlorides of sodium, potassium and calcium and discussed the physiological results obtained by the addition of each of these salts to physiological solutions. Shafer (1) studied the solution as a solvent for various salts. Fantus and Dyniewicz (2) suggested the use of Ringer's solution as a solvent for tannic acid in the treatment of burns. At the suggestion of the National Formulary Committee the following monograph was prepared for Ringer's solution, suitable for use as a solvent for hypotonic solutions to be used parentally or when chlorides of sodium, potassium and calcium in the tissues have been diminished.

LIQUOR PHYSIOLOGICALIS SALINIS

Solution of Physiological Salts

Ringer's Solution

Solution of Physiological Salts contains in 100 cc. not less than 0.84 or more than 0.88 Gm. of NaCl, not less than 0.025 and not more than 0.035 Gm. of KCl and not less than 0.030 or more than 0.036 Gm. of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$.

* The expense of this investigation was defrayed in part by a grant from the National Formulary Committee.

† Department of Pharmacology, School of Medicine, University of Maryland.