which are too small to be profitably used for this type of experimentation.

3. Roosters appear to be suitable for this type of study.

4. While the number of animals is undoubtedly very limited, it has been shown that of the four agents studied—allantoin, allantoin dipiperazine, 40% urea and physiologic salt solution—allantoin possesses the best healing action, urea the least.

5. Allantoin dipiperazine shows no better healing or granulating action than saline solution because the alkalinity of the solution destroys the allantoin, and thus the potency.

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A Study of Syrup of Hydriodic Acid*

By Howard Hopkins† and C. O. Lee‡

The solution of hydriodic acid from which the syrup was first prepared was unstable due to the presence of potassium iodate in the potassium iodide. Discoloration of the syrup in years gone by may have been due to the liberation of free iodine. With the coming of more highly purified chemicals its discoloration has been ascribed to other causes. Since about 1880 there have been numerous comments and discussions by many workers on how best to prepare, stabilize and store syrup of hydriodic acid.

Hydriodic acid has been used in medicine for more than a hundred years. Because of its unstable character it has been a problem. Many studies have been made with the view to finding a more suitable method for its preparation and preservation.

The first to report the use of hydriodic acid as a medicinal was Dr. Andrew Buchanan, Junior Surgeon to the Glasgow Royal Infirmary in 1837 (1, 2, 3). He gave detailed directions for the preparation of a dilute solution of the acid by the interaction of potassium iodide and tartaric acid. He preferred to use the acid, in the place of iodine, because he believed that it was less irritating to the stomach. He believed, also, that the stomach converted iodine into hydriodic acid, which reaction would be saved by administering the latter preparation. Buchanan proposed that the acid should be taken with starch gruel, arguing that any iodine which might be liberated would combine with the starch, and be less irritating.

Apparently the first attempt to stabilize the solution of hydriodic acid was reported in 1855 by Murdock (3). He said, "I find that hydriodic acid may be prevented from undergoing this decomposition when in the form of a syrup." He said further, "Assuming, therefore, that if a syrup can be prepared by Dr. Buchanan's solution that shall contain no free iodine, it will furnish the most suitable manner of obtaining this acid for medicinal purposes....It is necessary, however, to observe, as one of the conditions of success, that the iodide must be free from any trace of iodate of potash."

The U. S. Pharmacopœia IV (4) included a formula for diluted hydriodic acid, and

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directed that it be prepared by suspending iodine in water and passing hydrogen sulfide into the suspension. The U. S. Pharmacopœias V and VI offered no formulas for hydriodic acid. However the latter book (5) contained a formula for syrup of hydriodic acid, to be prepared according to the method of the U. S. Pharmacopœia IV. It was directed that the finished syrup be put into small, securely corked vials, and kept in a cool, dark place.

REPORTS OF EARLY INVESTIGATORS

In 1882 Godding (6) submitted a thesis to the Massachusetts College of Pharmacy in which were his findings concerning his efforts to stabilize syrup of hydriodic acid. He says, "It has been asserted that hyposulphite of sodium in solution has the advantage of preserving and reclaiming the acid when colored, but my experiments have failed to prove this assertion." Hence, this early attempt to prevent the discoloration that develops in syrup of hydriodic acid was not successful. Since Godding's time many investigators have given thought to this problem.

Cameron (7) proposed that the acid be prepared frequently, added to syrup, and stored in a cool, dark place. He said that syrup was the best vehicle available, since it prevented the discoloration so frequently observed in diluted hydriodic acid.

The first edition of the National Formulary (8), 1888, contained a formula for colorless syrup of hydriodic acid. The acid was prepared according to a modification of Buchanan's method, added to syrup, and preserved by the addition of potassium hypophosphite. So far as we know, this was the first time that this salt was proposed as a preservative for syrup of hydriodic acid.

In 1889 Beringer (9) was reported to have said that he had prepared a syrup of hydriodic acid which had remained stable for two years. Concerning the acid, England (10) said, "Since its introduction it has found a limited and varying demand among the medical profession." He attributed its lack of wide popularity to its instability. He claimed that honey, potassium hypophosphite, hypophosphorous acid and glycerin had little or no influence in retarding decomposition of the acid. He approved the National Formulary colorless syrup, but substituted syrupy glucose for the potassium hypophosphite. Raubenheimer (11) claimed that the National Formulary formula for colorless syrup of hydriodic acid was not so stable as his modification thereof containing glycerin.

The U. S. Pharmacopœia VII (12) did not include a diluted hydriodic acid, but the syrup was retained. The acid to be used in preparing the syrup was directed to be prepared according to a modification of Buchanan's method in preference to the hydrogen sulfide–iodine method, U. S. P. IV.

Syrup of hydriodic acid frequently developed an offensive odor upon standing. Haussmann (13) attributed this to the decomposition of ultramarine in acid media, hydrogen sulfide being liberated from ultramarine. Caspari (14) claimed that syrup of hydriodic acid kept well for some time if preserved in completely filled bottles and in a dark place.

In 1897 the U. S. Pharmacopœia Committee of Revision (15) reported that syrup of hydriodic acid was considered to be an unstable preparation and proposed that it be made extemporaneously from a strong solution of hydriodic acid and syrup.

Scoville (16) in 1899 reported that the more dilute the syrup, the more stable the preparation. He also reported that the substitution of glycerin for syrup yielded a stable product. Wentworth (17) confirmed this observation.

Peacock (18) proposed that the coloration observed in syrup of hydriodic acid might be due to caramelization of the sugar. Haussmann (19) concurred in this and suggested that the syrup be prepared by merely diluting the acid with simple syrup. He discounted the assertions of some that glycerin was an effective substitute for syrup. He indicated, however, that heat was of importance in producing discoloration of syrup of hydriodic acid. His investigations failed to reveal the presence of free iodine in discolored samples of syrup of hydriodic acid. The same samples were shown to

have lost none of their hydriodic acid content. Haussmann said, "A number of examinations revealed the fact that syrups at the time of preparation and after discoloration show identical percentages." He suggested the use of animal charcoal to remove color from the syrup, because it did not take out any of the hydriodic acid.

Wells (20) claimed that discoloration of syrup of hydriodic acid was, in nearly all cases, due to the action of the acid on impurities in the sugar or upon the sugar itself. In support of his contention he pointed out that other acid syrups, such as syrup of calcium lactophosphate, showed discoloration. He agreed with Scoville that a light syrup seemed more effective as a stabilizer than a heavy syrup. Inasmuch as heat caused syrup of hydriodic acid to discolor, he argued that it should be stored in a cool place.

Sieker (21) reported in 1902 that the discoloration or precipitate which developed in syrup of hydriodic acid was due to the decomposition of the sugars. He suggested that the syrup be prepared extemporaneously by diluting 1 vol. of 16% hydriodic acid with 15 vols. of simple syrup.

Koch (22) held to the view of Haussmann and Sieker that discoloration of the syrup of hydriodic acid was due to the decomposition of the sugar and agreed with Scoville and Wells that a light syrup was a more effective stabilizer than a heavy syrup.

The U. S. Pharmacopœia VIII (23) reintroduced diluted hydriodic acid. It was prepared according to a modification of Buchanan's method and the official syrup was made by mixing the diluted acid with simple syrup.

Francis (24) proposed that glycerin be used as the vehicle for hydriodic acid and that such a product be called "glycerole."

In 1908 Cook (25) showed that the use of a light syrup made a syrup of hydriodic acid which did not darken upon standing.

The U. S. Pharmacopœia IX (26) included diluted hydriodic acid and syrup prepared from it, the latter being 20% stronger than the formula in U. S. Pharmacopœia VIII.

In 1920 Snyder (27) reported for a committee of the American Drug Manufacturers Association to the effect that it believed that discoloration of syrup of hydriodic acid was due to the decomposition of a portion of the sugar. The committee recommended that the Pharmacopœia should state that the syrup should be made in small quantities and stored in a cool place.

Ewe (28) recommended a formula for syrup of hydriodic acid to contain 34 Gm. of sugar per 100 cc. of the syrup. He said that a higher concentration of sugar caused darkening and a lower one made an inferior product. Krantz (29) claimed that 72.5% of glycerin by volume was a better vehicle for hydriodic acid than syrup.

The U. S. Pharmacopœia X (30) included formulas for both diluted hydriodic acid and the syrup, the latter to be prepared from the diluted acid, sugar and distilled water.

ASSAYS OF SYRUP OF HYDRIODIC ACID

The first assay results to be reported upon the stability of hydriodic acid were those of Arny and his co-workers (31) in 1929. Their studies, which extended over nearly two years, showed that there was no loss of the iodide content in numerous samples of hydriodic acid made using glycerin, reducing sugars, and syrup in the formulas.

Snyder (32) expressed the view, in 1930, that syrup of hydriodic acid was a drugstore product that every pharmacist should be prepared to compound extemporaneously. He suggested that the syrup prepared from glycerin developed disagreeable odors which were attributed to the presence of impurities in the glycerin. Ewe (33) pointed out that glycerin would be a satisfactory vehicle for hydriodic acid if it were free from butyric acid. He believed that discoloration of the syrup was due to caramelization of the sugar by excessive acidity. Heyl (34) meanwhile reported that a reduction of the hypophosphorous content did not prevent precipitation and discoloration of the syrup. Saalbach (35) claimed that discolored syrups could be made colorless upon their exposure to direct sunlight. He further suggested that the Pharmacopœia should state

that it be stored in clear glass bottles exposed to the sunlight.

In 1931 Husa (36) reported the first of a series of studies upon hydriodic acid and syrup of hydriodic acid. It was his belief, at that time, that the syrup should be prepared in the drugstore as needed. In the same year Arny (37) and his co-workers reported their studies upon the deterioration of certain medicaments under the influence of various conditions of light and storage. They had the following to say, "The . . . products, all of the quality prescribed by the U. S. P. X or U. S. P. IX, maintained their full halogen content, even after one year of exposure to direct daylight or to diffused light. . . . The iodide preparations did darken in direct daylight, all samples darkening between the fourth and sixth month of exposure except in the case of flint glass and the amber containers where the sample of the acid remained colorless for about twelve months." They declared that hypophosphorous acid was mistakenly added as a preservative.

Husa and Klotz (38) attacked the problem from a new angle in 1935. They used different sugars in preparing the syrup of hydriodic acid and concluded that its discoloration was due to the decomposition of levulose formed upon hydrolysis of the sugar.

They were able to prepare a superior syrup by using 700 Gm. of dextrose (commercial or a chemically pure grade) per liter of the finished product. It remained clear and colorless even upon continued exposure to an oven temperature of 50° C. for three months.

EXPERIMENTAL

The experimental part of this study follows closely that of Husa and Klotz. The sugars sucrose and glucose were used alone and in combination. Glycerin and alcohol were used in the same way in part of the formulas, replacing an equal volume of water in each case.

Each sample of syrup was divided into three 2-oz. portions in regular prescription bottles. Each portion was stored differently; namely, (*a*) in a warm place (about 40° C.), (*b*) in a refrigerator and (*c*) at room temperature. Observations were made at frequent intervals. The samples were assayed at the time of preparation or soon thereafter, and at the end of the test period.

Twenty formulas were prepared as indicated in Table I.

The U. S. P. XI assay procedure was followed except that a smaller quantity of syrup than that

TABLE I

	Formulas and Variations			
	1[a]	2	3	4
Formula A				
Diluted HI	23.4 cc.	23.4 cc.	23.4 cc.	23.4 cc.
Sucrose	81.0 Gm.	81.0 Gm.	81.0 Gm.	81.0 Gm.
Glycerin	.....	18.0 cc.	.....	18.0 cc.
Alcohol	.....	.....	19.0 cc.	19.0 cc.
Dist. water, *q. s. ad.*	180.0 cc.	180.0 cc.	180.0 cc.	180.0 cc.
Formula B				
Diluted HI	23.4 cc.	23.4 cc.	23.4 cc.	23.4 cc.
Dextrose	126.0 Gm.	126.0 Gm.	126.0 Gm.	126.0 Gm.
Glycerin	.....	18.0 cc.	.....	18.0 cc.
Alcohol	.....	.....	19.0 cc.	19.0 cc.
Dist. water, *q. s. ad.*	180.0 cc.	180.0 cc.	180.0 cc.	180.0 cc.
Formula C				
Diluted HI	23.4 cc.	23.4 cc.	23.4 cc.	23.4 cc.
Glucose	78.3 cc.	78.3 cc.	78.3 cc.	78.3 cc.
Glycerin	.....	18.0 cc.	.....	18.0 cc.
Alcohol	.....	.....	19.0 cc.	19.0 cc.
Dist. water, *q. s. ad.*	180.0 cc.	180.0 cc.	180.0 cc.	180.0 cc.
Formula D				
Diluted HI	23.4 cc.	23.4 cc.	23.4 cc.	23.4 cc.
Sucrose	64.8 Gm.	56.7 Gm.	40.5 Gm.	24.3 Gm.
Dextrose	25.2 Gm.	37.8 Gm.	63.0 Gm.	88.2 Gm.
Dist. water, *q. s. ad.*	180.0 cc.	180.0 cc.	180.0 cc.	180.0 cc.
Formula E				
Diluted HI	23.4 cc.	23.4 cc.	23.4 cc.	23.4 cc.
Sucrose	86.4 Gm.	72.0 Gm.	61.2 Gm.	54.0 Gm.
Dist. water, *q. s. ad.*	180.0 cc.	180.0 cc.	180.0 cc.	180.0 cc.

[a] Formula 1-*A* official in U. S. P. XI.

specified was taken for a sample. Ten cubic centimeters of the syrup and 40 cc. of distilled water were placed in a 250-cc. Erlenmeyer flask and mixed well, 25.00 cc. of 0.1 *N* silver nitrate solution was added next, and lastly 2 cc. of nitric acid; the mixture was then placed on a water bath and heated until the precipitate acquired a yellow color or until the supernatant liquid became clarified. The excess silver nitrate was then titrated with 0.1 *N* ammonium thiocyanate solution. Ferric ammonium alum was used as the indicator. Duplicate assays were run in each instance. The assay results are given in Table II.

TABLE II

Date of Assay, 1941	Storage Condition	Grams of HI per 100 Cc. of Syrup 1	2	3	4
Formula A					
April 12	Cold	1.390	1.407	1.381	1.388
	Room	1.357	1.406	1.383	1.394[a]
	Warm	1.380	1.395	1.371	1.388
May 14	Cold	1.401	1.413	1.386	1.407
	Room	1.344	1.394	1.387	1.403
	Warm	1.367[a]	1.397	1.361	1.389
Formula B					
April 12	Cold	...	...	...	...
	Room	...	...	...	...
	Warm	...	...	...	...
May 14	Cold	1.386	1.501	1.373	1.377
	Room	1.382	1.500	1.372	1.385
	Warm	1.383	1.498	1.360	1.386
Formula C					
April 17	Cold	1.674	1.472	1.674	1.627
	Room	1.670	1.496	1.528	1.528
	Warm	1.528	1.496	1.528	1.550
May 14	Cold	1.542	1.500	1.542	1.544
	Room	1.564	1.486	1.550	1.542
	Warm	1.542	1.500	1.546	1.563
Formula D					
April 12	Cold	1.458	1.496	1.670	1.496
	Room	1.458	1.496	1.670	1.496
	Warm	1.458	1.496	1.670	1.496
May 14	Cold	1.489	1.516	1.558	1.516
	Room	1.462	1.518	1.500	1.500
	Warm	1.475	1.489	1.558	1.516
Formula E					
April 12	Cold	1.350	1.375	1.376	1.378
	Room	1.350	1.375	1.376	1.378
	Warm	1.350	1.375	1.376	1.378
May 14	Cold	1.377	1.382	1.410	1.377
	Room	1.374	1.377	1.402	1.365
	Warm	1.361	1.378	1.377	1.361

[a] A single determination.

From these results, it is evident that there is no significant loss of the iodide content when syrup of hydriodic acid is stored at room temperature, in the cold, or in a warm place. The conditions of storage, however, have a marked effect on the discoloration of the syrup. In general, it may be said that those samples stored in a warm place darkened quickly and to a marked degree, whereas those stored at room temperature darkened less quickly and to a lesser degree. The samples of syrup stored in the refrigerator did not, in general, darken at all.

Formula *A* gave syrups that darkened quickly and intensely. They contained sucrose alone as the sugar. Formula *E*, containing only sucrose as the sugar, produced syrups that gave increasing discoloration with increasing sugar concentration. Formulas *C* and *D* produced syrups that discolored somewhat. Formula *B* gave a syrup of hydriodic acid that remained clear and colorless in all instances. It was made with dextrose. These results coincide with those of Husa and Klotz. Their proposal to use dextrose instead of sucrose in preparing syrup of hydriodic acid seems to us to be sound.

SUMMARY AND CONCLUSIONS

1. The early history and development of the use of hydriodic acid and syrup of hydriodic acid have been reviewed.

2. Assay results are given showing that discoloration, if it occurs, does not affect the iodide concentration in syrup of hydriodic acid.

3. In general, the rate of discoloration and the intensity of the color produced increase as the storage temperature increases.

4. When sucrose alone is used as the sugar in preparing syrup of hydriodic acid, the rate of discoloration and the intensity of the color produced increase with increasing sucrose concentration.

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A Study of Isotonic Solutions*

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There is an increasing interest in the use of medicinal solutions having the same osmotic pressure as the body fluids or bearing a definite relation thereto. Originally the main interest in this field was concerned with parenteral solutions, but recently considerable attention has been given to adjustment of the osmotic pressure of collyria and nasal preparations.

Hypotonic solutions may be made isotonic by addition of more of the drug or some other substance. The proportion of substance to be added may be determined experimentally or calculated mathematically. In the present paper, the value, limitations and accuracy of these methods are discussed and new experimental data are presented.

THEORETICAL

The idea of isotony is connected with the knowledge of osmotic pressure, which was first observed by Nollet in 1748. The term "osmosis" was introduced by Dutrochet.

Plasmolytic Method.—In 1888, De Vries found that when certain vegetable cells were placed in solutions containing 7.5% or more of sucrose, water passed out from the cells, which then contracted away from the sheath of cellulose. This phenomenon is called plasmolysis. By varying the concentration of the solution it is possible to determine the concentration at which plasmolysis ceases or is barely detectable; such solutions are said to be isotonic with the cell sap, *i. e.*, capable of producing the same pressure in the cell. The abnormal osmotic pressure of salt solutions was first observed by De Vries, who introduced the term "isotonic coefficient" to express the degree of deviation from normal. The plasmolytic method is not trustworthy for accurate measurements, as it is subject to numerous errors, such as reaction of the cell contents with the substances studied, exosmosis of cell contents, etc.

Hemolytic Method.—The hemolytic method, which was developed in 1890 by Hamburger, consists in the determination of the concentration which produces laking of red blood corpuscles, which are semipermeable to solutions of most substances except urea and ammonium salts. Wokes (1) determined the ratio *isotonic concentration/hemolytic concentration*, and found that this ratio was different for different substances. Some of the ratios were as follows: sodium chloride, 2; dextrose, 2.9; sodium bicarbonate, 3.1. Boric acid was hemolytic in all concentrations. Wokes concluded that if a solution diluted with half its volume of distilled water is hemolytic, it is not isotonic and should not be used for injection.

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