A comparative study on the effect of heat, hydrogen-ion concentration, cathions and anions, on 2.5 per cent solutions of bismuth sodium citrate in water and in ethyleneglycol indicates that in the ethyleneglycol solution, the bismuth sodium citrate is less dissociated, and, therefore, more stable.

The precipitant action of these solutions on serum and egg-albumin solutions indicates the same fact.

Ethyleneglycol solutions have the properties of hypertonic solutions, and from ethyleneglycol solutions, the bismuth diffuses more rapidly than from aqueous solutions.

STUDIES ON ALKALI BISMUTH TARTRATES FROM THE CHEMICAL AND BIOLOGICAL STANDPOINT.*

BY A. E. JURIST AND W. G. CHRISTIANSEN.

Although many publications have appeared concerning the preparation of alkali bismuth tartrates and the clinical value of them in the treatment of syphilis, little information is available regarding the behavior of alkali bismuth tartrates in aqueous solutions containing various stabilizing substances, such as buffers, sugars of different types, salts intended to aid absorption, protective colloids, etc. In addition to the results of this series of stability studies, a rather extensive set of experiments will be presented relating to the toxicity, absorption and local action (irritation at site of injection or other local reactions) of alkali bismuth tartrates when aqueous solutions of the latter are injected intramuscularly; particular reference will be made to the type of solution used. Also, the results of some rough p_H determinations using an indicator will be shown to have a direct relation to the stability of these solutions.

It was found that a very good estimate of the stability of a solution could be made by heating sealed ampuls of the solution at 105° C. and making periodic observations thereon. For the most part any solution which survived the heat-test for one-half hour or more was sufficiently stable to remain unchanged for the length of time required to make the biological studies desired. Also it was found that the sterilized and unsterilized solutions were on a par as regards stability; solutions used for the biological tests were sterilized by passage through a Berkefeld filter prior to ampuling. All solutions were made up to contain approximately 0.05 Gm. of bismuth metal per cc. or about 7% of the alkali bismuth tartrate. The data given in the following table describes most of the solutions prepared and the stability tests run on them. Unless otherwise stated the compound used was potassium tribismuth tartrate. As will be shown the difference between sodium tribismuth tartrate and potassium tribismuth tartrate was negligible.

TABLE I.—BISMUTH TARTRATE SOLUTIONS AND THEIR STABILITY.

Number.	Material added to the solution.	Stability at 105° C.	Stability in storage at room temperature.
1	5% Sucrose	Opalescent in 120 minutes	Clear light brown color
2	5% Sucrose and 2% benzyl alcohol	Opalescent in 120 minutes	Unchanged six mos.
3	10% Sucrose	Opalescent in 120 minutes	Clear light brown color
4	10% Sucrose and benzyl alcohol 2%	Opalescent in 120 minutes	Unchanged six mos.

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* Scientific Section, A. PH. A., Baltimore meeting 1930.

5	25% Sucrose	Opalescent in 250 minutes	Clear light brown color
6	25% Sucrose and 2% benzyl alcohol A	Opalescent in 250 minutes	Unchanged six mos.
7	25% Sucrose and 2% benzyl alcohol B	Opalescent after 30 minutes	Precip'd in 90 days
8	25% Sucrose and 2% benzyl alcohol and 2% sodium taurocholate		Precip'd in 90 days
9	25% Sucrose and 2% benzyl alcohol and 2% triethylamine oleate		Precip'd Bismuth Oleate in 10 minutes
10	12.5% Lactose and 2% benzyl alcohol	Precipitated in 30 minutes	Clear but yellow in 90 days
11	5% Acacia and 2% benzyl alcohol		Precip'd in 10 min.
12	20% Glycerin and 2% benzyl alcohol	Unchanged in 24 hours	Unchanged in 8 mos.
13	25% Sucrose and 0.4% tartaric acid	Decomposed in 30 minutes	Cloudy, jelled in 60 days
14	25% Sucrose and 15% sodium thiosulphate	Precipitate of Bi_2S_3 in 30 minutes	
15	25% Sucrose and 10% sodium citrate	Precipitated bismuth citrate in 30 minutes	
16	25% Sucrose and CO_2 bubbled in until opalescent	Precipitated bismuth hydroxide in 10 minutes	
17	25% Sucrose and 0.6% potassium acid tartrate	Cloudy after 105 min.	Clear and colorless after 60 days
18	25% Sucrose and 2% benzyl alcohol and 5% glycerin	Precipitated in 85 minutes	Clear and colorless after 60 days
19	25% Sucrose and 2% benzyl alcohol (sodium tribismuth tartrate)	Precipitated in 140 minutes	
20	10% Sodium citrate and 2% sodium taurocholate	Precipitated in 10 minutes	
21	10% Glycerin and 10% sodium citrate	Precipitated in 10 minutes	
22	10% Glycerin and 2% sodium taurocholate	Precipitated in 10 minutes	
23	Colloidal solution in 14% of hydrolyzed gelatin	Jells and precipitates in 45 minutes	Cloudy and jelled in 2 months
24	10% Potassium gluconate	Immediate precipitate at room temperature	
25	10% Dextrose	Decomposed in less than one hour	
26	10% Levulose	Decomposed in less than one hour	
27	10% Glucose	Decomposed in less than one hour	Clear but brown in 90 days
28	10% Sorbitol	Jelled in one hour	
29	25% Sucrose	Unaltered in 30 minutes	

NOTE: All solutions 1–6 made with one lot of the bismuth tartrate and 7–29 made with a second lot.

The results given above will be discussed in different groups depending on the character of the dissolving medium. It was found that instability showed itself in different ways. Thus, solutions which were excessively alkaline deposited bismuth hydroxide; wherever the possibility existed an insoluble bismuth salt precipitated, *e. g.*, in the cases where alkali salts of organic acids were used; all the reducing sugars caused the liberation of metallic bismuth; some solutions formed stiff jells; and where sulphur was present bismuth sulfide precipitated.

The first group of substances to be considered will be the sugars. Of these dextrose, levulose and glucose are reducing sugars containing either the aldehyde or the ketone group. In every instance a copious precipitate of metallic bismuth was formed at 105° C. and only the glucose solution remained clear when stored at room temperature but it turned dark brown in color. Sorbitol, an alcohol sugar which contains no reducing groups, did not liberate bismuth metal but jelled quite rapidly. The polysaccharides, lactose and sucrose, gave solutions which remained clear on storage at room temperature, but the lactose solution discolored whereas the 25% sucrose solution did not. The more dilute sucrose solutions were less stable than the 25% solution, and benzyl alcohol had no effect on the stability of these solutions. There was no appreciable difference between the stability of sodium tribismuth tartrate and potassium tribismuth tartrate solutions in 25% sucrose. From an examination of the results with sugar solutions it is apparent that the most stable solutions were made with sucrose and that only the sucrose solutions showed any stability when heated.

Various attempts were made to either increase the speed of absorption of sucrose solutions or buffer them by the addition of various salts of organic acids. These included sodium taurocholate, sodium citrate, tartaric acid, potassium acid tartrate and triethanolamine oleate. Sodium taurocholate, sodium citrate and triethanolamine oleate all precipitate the insoluble bismuth salts of the organic acids very rapidly when heated. The solution buffered with tartaric acid jelled, but the solution prepared with potassium acid tartrate was more stable than the corresponding potassium tribismuth tartrate solution in 25% sucrose without potassium acid tartrate. A sucrose solution made with sodium thiosulfate was unstable; it precipitated bismuth sulphide quite readily. Of these solutions the most stable was the potassium acid tartrate solution in 25% sucrose, and it was more stable than any of the other sugar solutions.

A solution made with glycerin and benzyl alcohol, using no sucrose, was prepared and this was extremely stable, in fact the most stable solution prepared. Consequently an attempt was made to stabilize a 25% sucrose.solution by adding 5% of glycerin but this did not improve the stability of the sucrose solution at all. Also attempts were made to prepare aqueous solutions using either glycerin and sodium taurocholate or sodium citrate, or sodium taurocholate and sodium citrate but no sucrose. These solutions all precipitated bismuth salts of the organic acids immediately showing that glycerin could not be used to stabilize them. Similarly a potassium gluconate solution with potassium tribismuth tartrate immediately precipitated bismuth gluconate. Of all these solutions prepared without sucrose only the 20% glycerin solution was stable.

The last type of solution to be discussed is the one in which colloidal bismuth tartrate is prepared or protective colloids are used. In one instance a 25% sucrose solution was just neutralized to phenolphthalein by bubbling in carbon dioxide but this was unsatisfactory because the solution, initially hazy, soon precipitated, serving once more to emphasize the sensitivity of alkali bismuth tartrates to carbon dioxide. Then a solution of the bismuth tartrate in 5% acacia was made up; immediate precipitation of the insoluble arabic acid bismuth salt occurred. The most promising of the colloidal solutions was prepared by dissolving 7 Gm. of potassium tribismuth tartrate in 100 cc. of a 14% hydrolyzed gelatin solution and then neutralizing to phenolphthalein with carbon dioxide. This gave a fairly stable colloidal bismuth tartrate solution which was opalescent but did not precipitate. On storage or heating, however, it set to a firm jell thus eliminating it as a satisfactory type of solution. All the colloidal solutions were unsatisfactory.

From the stability standpoint, only the 25% sucrose solutions, the 25% sucrose solution buffered with potassium acid tartrate, and the 20% glycerin solution were sufficiently stable for satisfactory storage. These three solutions are of increasing stability in the order named. The fact that the solution containing the potassium acid tartrate was more stable than one without it brings up the question of p_H in relation to the stability of these solutions (1). No suitable electrode such as the quinhydrone electrode was available so recourse was had to indicators. Indigo carmine (2) faded badly but was nevertheless sufficiently stable to show any significant differences between the various solutions in the highly alkaline range of these substances. A series of results is given in the following table showing clearly the relation between p_H and stability.

TABLE II.—p_H DETERMINATIONS ON ALKALI METAL TRIBISMUTH TARTRATES.

Product.	Solution.	p_H.
Potassium tribismuth tartrate**	25% Sucrose	12.3
Potassium tribismuth tartrate***	25% Sucrose	11.8
Potassium tribismuth tartrate*	25% Sucrose	11.2
Potassium tribismuth tartrate**	25% Glycerin	11.0
Potassium tribismuth tartrate**	25% Sucrose and potassium acid tartrate	11.2
Potassium sodium bismuth tartrate	10% Glucose	12.3

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* Lot A. ** Lot B. *** Lot C.

These results are very significant in explaining why the solution of batch A in 25% sucrose was more stable than that of B and why C in turn gave more stable solutions than B. Thus, the most stable solutions, namely, those of batch B in 20% glycerin and in 25% sucrose containing 0.6% of potassium acid tartrate, have a p_H of 11.0 and 11.2, respectively; the latter results were also obtained with lot A in aqueous sucrose without potassium acid tartrate. This then explains the superior stability of solutions of potassium tribismuth tartrate A over B since the latter without a buffer gives a p_H one unit higher than the most stable solution. Also compound C having a lower p_H than B is more stable. These results do not apply to potassium sodium bismuth tartrate since this compound, having two atoms of alkali metal to one of bismuth and one of tartaric acid, should give a higher p_H and still be stable at that p_H.

The results indicate that the only water solutions of alkali tribismuth tartrates which are satisfactory from the standpoint of stability are made up in 25% sucrose and buffered, if necessary, to a p_H of 11.0 to 11.2 with potassium or sodium acid tartrate depending on the alkali metal present in the bismuth compound. No difference from the stability standpoint can be detected between the monosodium tribismuth tartrate and the potassium tribismuth tartrate used in most of these experiments.

The next tables will give the results of animal studies on absorption, toxicity and local irritation of a number of the preparations discussed in the foregoing. Absorption and toxicity tests were run on albino rats, but the local effects at the site of injection were studied with dogs since they are more sensitive and are more likely to show any differences which might exist between different solutions. Also some comparison will be made between water solutions and oil suspensions as regards toxicity and absorption.

The following table summarizes the results of studies of the local effects produced by intramuscular injection of alkali bismuth tartrates in dogs. The results show the comparative value of different media for preparing these solutions. The dosage was 1 cc. of solution containing approximately 0.05 Gm. of bismuth per cc. The three factors considered are pain, swelling and lump formation, pain is graded as slight, definite (sensitive to contact), and decided (very sensitive to contact). Swelling means general swelling in the area surrounding the site of injection. Lump formation refers to the formation of lumps at the site of injection of varying size, persisting from one to fourteen days and being hard to the touch. In studying the latter type of local reactions the table indicates the maximum reaction observed in a majority of the animals injected with the material being tested.

TABLE III.—LOCAL REACTIONS PRODUCED IN DOGS BY INTRAMUSCULAR INJECTIONS OF ALKALI BISMUTH TARTRATES.

Preparation.	Swelling.	Pain.	Lump formation (size).
Potassium sodium bismuth tartrate	Slight	Moderate	Hen egg
Sodium tribismuth tartrate	Slight	Slight	Hen egg
27	Slight	Slight	Pea size
6	Moderate	Slight	Hen egg
7	Slight	Decided	Pea size
8	Slight	Slight	Pigeon egg
12	Very slight	Slight	Pigeon egg
29	Very slight	Decided	Hen egg
18	Very slight	Definite	Hen egg
19	Very slight	Definite	Hen egg
17	Very slight	Slight	Hen egg
23	Very slight	Slight	Hen egg

NOTE: The numbers in the above refer to those in Table I.

No effects such as are described in this table were noted when the diluent was injected without the alkali bismuth tartrate except that sodium taurocholate caused slight lump formation with no local pain or swelling. Glycerin caused necrosis in 25% of the experiments in which it was used alone.

The results show the formation of large lumps in every case but one. All injections were followed by some local swelling and varying degrees of pain, but lump formation was the most persistent and severe reaction. Since it has been found that the diluents without the alkali bismuth tartrates did not cause these severe local reactions it is apparent that the alkali bismuth tartrates alone are responsible. It is not surprising then that all the solutions appear to give similar reactions showing only minor variations. In view of the above results, the severe local reactions sometimes encountered in human beings injected with alkali bismuth tartrates are not surprising but, rather, to be expected. The results do not indicate that from the biological standpoint any one medium is superior to any other for preparing aqueous solutions of the alkali bismuth tartrates.

The results of the toxicity and absorption studies are given in the following table. Owing to the nature of the compounds tested it is impossible to obtain an accurate absolute toxicity but it is possible to get an approximate result from growth curves which form the basis for the results given. It is unnecessary to indicate more than complete or incomplete absorption, because a single injection of an aqueous solution is usually almost completely absorbed within the first 72 hours after injection. In cases of incomplete absorption the results were variable although extended over a period of about sixty days or more. Inasmuch as other published results have demonstrated that oil suspensions are not completely absorbed whereas the aqueous solutions usually are, the work on oil suspensions was not extensive.

TABLE IV.—TOXICITY AND ABSORPTION OF ALKALI BISMUTH TARTRATES ON ALBINO RATS.

Description of material tested.	Absorption.	Toxicity (approx.), mg. per Kg. of body wt.
Sodium tribismuth tartrate in 10% glucose	Complete	600
Sodium tribismuth tartrate in 25% sucrose and 2% benzyl alcohol	Complete	600
Potassium tribismuth tartrate in 10% glucose	Complete	600
Potassium tribismuth tartrate A in 25% sucrose and 2% benzyl alcohol	Complete	600
Potassium tribismuth tartrate B in 25% sucrose and 2% benzyl alcohol	Complete	600
Potassium tribismuth tartrate B in 25% sucrose and 2% benzyl alcohol and 2% sodium taurocholate	Complete	600
Potassium tribismuth tartrate B in 25% sucrose	Complete	600
Potassium sodium bismuth tartrate in 10% glucose	Complete	50
Sodium tribismuth tartrate in oil suspension	Incomplete	600
Potassium tribismuth tartrate in oil suspension	Incomplete	600

The results show no variations in the toxicity and absorption of the sodium and potassium tribismuth tartrates whether the solution is in 10% glucose, 25% sucrose or oil suspension. The addition of 2% of sodium taurocholate to the 25% sucrose solution or the addition of benzyl alcohol seem to be without effect on these characteristics. Sodium taurocholate solution accelerated the absorption initially, but the rate was not sustained owing, perhaps, to tissue damage which was more extensive than usual and which was probably due to over-rapid absorption of the toxic bismuth compound. The potassium sodium bismuth tartrate was as well absorbed as the others, but it was much more toxic than the sodium or potassium tribismuth tartrates. The oil suspensions were not completely absorbed. There was no significant difference in toxicity between the potassium and sodium tribismuth tartrates. However, owing to the incomplete and much slower absorption of the oil suspensions, the toxicity of sodium and potassium tribismuth tartrates in this form may be slightly lower than in aqueous solution, but again the difference is slight.

The results of these studies show that stable solutions of the alkali bismuth tartrates can be prepared using glycerin or 25% sucrose properly adjusted for p_H when necessary. The biological tests favor the latter type of solution since the glycerin alone can produce reactions which sucrose does not produce. The use of the alkali bismuth tartrates in aqueous solution is accompanied by marked local reactions including pain, local swelling and lump formation at the site of injection. These reactions are due in each case to the alkali bismuth tartrate and are in no way related to the nature of the aqueous media in which they are dissolved. Therefore, it would appear that in spite of their low toxicity and ready absorption aqueous solutions of the alkali bismuth tartrates are far from satisfactory therapeutic agents in syphilis; the severe local reactions which they produce on intramuscular injection in animals are almost certain to be paralleled in man. It does not appear to be possible to materially reduce these local reactions by modifying the aqueous media in which the compound is dissolved.

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A PHYSICO-CHEMICAL METHOD OF MEASURING THE ACTIVITY OF PEPSIN. PART I—A PRELIMINARY STUDY.*,†

BY EDWIN R. THEIS.

The usual procedure in measuring the activity of pepsin, has been to allow the enzyme to act in acid medium upon the substrate (coagulated egg albumin) at a more or less constant temperature for several hours and then measuring the amount of digestion by one of several methods. While this method is being used universally, it lacks many of the real essentials of being completely quantitative in nature and the personal error is in many cases rather large.

The writer, in studying the nature of pepsin and the kinetics of enzyme action in detail, came upon a physico-chemical method that may be of more than passing interest. It has been shown[1] in former work that when any protein is placed in water, a net contraction in volume of the entire system results, in other words the protein hydrates. As hydration proceeds, the net contraction of the system continues until an equilibrium condition is attained. This contraction of the system is the result of an internal compression of the absorbed liquid within the protein. It has been further shown[2] that if an enzyme, such as pepsin, is added to the liquid medium, hydration proceeds as before, but a temporary equilibrium results at an earlier period, at which time hydration ceases and dehydration

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† Scientific Section, A. PH. A., Baltimore meeting, 1931.

[1] *Ind. Eng. Chem.*, 21 (1929), 377; 22 (1930), 64.

[2] *Ibid.*, 22 (1930), 64.