have only a very slight advantage over higher temperatures. Thus the refrigerator is of very little use as a means of storage. The replacing of the oxygen with a non-oxidizing gas, carbon dioxide, inhibits deterioration to a slight degree.

Sealing with paraffin to have the container air tight, proved useless since the results show some loss of potency whether the container was sealed or unsealed.

It is to be noted that the assay of all the tinctures at the end of a year of storage shows them to be of approximate equality, regardless of the original activity. It would seem that after a period of time an equilibrium is reached when no further destruction of glucosides occurs, disregarding the method of manufacture and the conditions of storage. This may be due to an inactivation of the added alkali or acid, or may be due to the fact that some of the glucosides are stable and not destroyed while others are labile and readily broken up in an acid or alkali medium. This process probably occurs in the normal tincture also, since there is comparable loss in activity.

The exact mechanism involved in this process of deterioration is at present not known but is the subject of further investigation.

VII. CONCLUSIONS.

1. Increasing or decreasing the p_H of the menstruum in making tincture of digitalis produces an inferior product.

2. Storage at room temperature, or 6° C., has no differential effect on the keeping qualities of this preparation.

3. Tincture of digitalis stored in sealed or unsealed containers deteriorates to the same degree.

4. Displacing the air in a container with carbon dioxide has but a slight inhibitory action on the deterioration.

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STUDIES ON THE PREPARATION, TOXICITY AND ABSORPTION OF BISMUTH COMPOUNDS. I. BISMUTH SALTS OF FATTY ACIDS.*

BY W. M. LAUTER, A. E. JURIST AND W. G. CHRISTIANSEN.

While a great many clinical reports have been made describing the results obtained with bismuth salts of fatty acids in the treatment of syphilis, very little has been published concerning the results obtained in carefully controlled experimental studies on animals. Most of the results so far published are concerned chiefly with the excretion of bismuth giving very little accurate data concerning the relative toxicity and absorption of these compounds. The purpose of these studies is to compare these substances with a number of other types of bismuth

* Scientific Section, A. PH. A., Toronto meeting, 1932.

compounds from the standpoint of toxicity and absorption and also to describe the methods used in preparing them. Some information on the subject is already available in the publications of Ritz (1), Von Oettingen (2), and Von Oettingen and Sollmann (3).

In this paper a number of bismuth salts of fatty acids of different types will be compared from the standpoint of absorption, but the results obtained with bismuth compounds of other types will be considered in subsequent papers. The fatty acids which were used in preparing the bismuth compounds include saturated acids with varying numbers of carbon atoms, unsaturated acids, hydroxy acids, dibasic acids and fatty acids containing a ring structure.

The method used in the study of these fatty acid derivatives of bismuth was to prepare olive oil suspensions or solutions of known bismuth concentration with or without the addition of an excess of the fatty acid from which the bismuth salt was prepared. These suspensions or solutions were then injected intramuscularly into albino rats. The toxicity of the compound was then estimated from the growth curves of the animals while the absorption was determined by killing the animals after a period of 27 to 35 days after injection and determining by analysis any unabsorbed bismuth still remaining at the site of injection.

The results of the absorption studies on these substances are given in the following table. The data includes the nature of the medium in which they were injected, the bismuth concentration, and the total bismuth injected into a single animal.

TABLE I.

Compound Injected.	Nature of Medium for Injection.	Dosage Mg. Bi/Cc.	Total Bismuth Injected in Mg./Kg. Body Weight.	Per Cent Absorption.
Bismuth oleate	Olive oil	50	190	22
Bismuth myristate	Olive oil	20	95	19
Bismuth laurate	Olive oil	50	250	17
Bismuth palmitate	Olive oil	20	100	27
Bismuth stearate	Olive oil	25	115	36
Bismuth isolinolate	Olive oil	37.5	200	48
Oleo-bi	Olive oil	50	235	16
Bismuth azelate	Olive oil and azelaic acid	50	160	66
Bismuth ricinoleate	Olive oil and ricinoleic acid	50	165	93
Bismuth azelate	Olive oil	50	165	78
Bismuth α-octyl-α-hydroxysebacate	Olive oil	50	165	47
Bismuth 9,10 - dihydroxystearate	Olive oil	30	180	50
Basic bismuth chaulmoograte	Olive oil	35	185	58
Bismuth chaulmoograte	Olive oil and chaulmoogric acid	50	175	52
Bismuth ricinoleate	Olive oil	30	185	40
Bismuth hydnocarpate	Olive oil	40	175	50
Bismuth ricinoleate	Olive oil and ricinoleic acid	30	200	78
Bismuth ricinoleate	Olive oil and ricinoleic acid	30	200	48

These results show that the bismuth compounds of fatty acids are, as a rule, only slightly absorbed. Even in a period of four to five weeks more than 90% of the

bismuth was absorbed in only one instance, namely, bismuth ricinoleate in olive oil with additional ricinoleic acid. In eight instances more than 50% of the bismuth was absorbed. The average absorption was 47% considering all the substances tested. It is impossible to state from these results that any particular type of fatty acid gives a bismuth salt which is more readily absorbed than any other type. It is, however, quite apparent that absorption was unsatisfactory in every instance. The poor absorption obtained with these water-insoluble preparations contrasts strongly with the rapid and complete absorption obtained with water-soluble bismuth compounds. Owing to the very slow and irregular absorption obtained with these compounds no accurate determination of their relative toxicity can be made but since the lowest dosage used was 95 mg. of bismuth per Kg. body weight in the case of bismuth myristate and the highest dosage used was 250 mg. per Kg. of body weight in the case of bismuth laurate and since the average dosage for all the compounds tested was 174 mg. of bismuth per Kg. of body weight, it is quite evident that they are relatively low in toxicity.

These results clearly indicate that the bismuth salts of the fatty acids are relatively unsatisfactory therapeutic agents because their irregular and slow absorption would tend to give variable and unreliable clinical results.

EXPERIMENTAL PART.

The preparation of the various bismuth salts used in these solutions or suspensions is briefly described. It was found advisable in all of these preparations to remove any unreacted free acid by washing with acetone. When alcohol was used in place of acetone it was found that the bismuth compounds were decomposed so that the end product was usually a mixture of monobasic and dibasic bismuth salts, free fatty acid and bismuth hydroxide.

PREPARATION OF THE BISMUTH SALTS OF UNSATURATED FATTY ACIDS.

Bismuth Oleate.—28 Gm. of oleic acid were dissolved in 170 cc. of 95% alcohol, 30 cc. of water and 3.98 Gm. of sodium hydroxide. To this was added a solution of 16 Gm. of bismuth nitrate pentahydrate in 13 Gm. of mannite and 100 cc. of water in a fine stream with constant agitation. A white precipitate formed which quickly coagulated to a gummy mass. After several hours standing the supernatant liquid was decanted and the white mass was washed three times with water, being allowed to stand over night with the third washing. The solid was then dried *in vacuo* over caustic soda for 48 hours. The pale cream-colored paste thus obtained was not pure so that it was extracted with acetone to remove any uncombined fatty acids. The residue from this extraction was then washed three times with acetone and dried at 95° C. The yellowish white powder so obtained was a dibasic bismuth oleate.

Calcd. for $C_{17}H_{33}CO_2Bi(OH)_2$: Bi, 39.75%; $C_{17}H_{33}CO_2$, 53.76%. Found: Bi, 40.60%; $C_{17}H_{33}CO_2$, 48.30%.

Oleo-Bi.—This product was examined by suspending the contents of an ampul in acetone. The white solid which separated was collected in a centrifuge and washed with acetone. When dry the product was identical with the bismuth oleate described above.

Calculated for $C_{17}H_{33}CO_2Bi(OH)_2$: Bi, 39.75%; $C_{17}H_{33}CO_2$, 53.76%. Found: Bi, 39.12%; $C_{17}H_{33}CO_2$, 47.02%.

Bismuth Linolinate.—The linolinic acid used in this preparation was obtained from linseed oil. It contained some oleic, linoleic and isolinoleic acids. The bismuth compound which was prepared in the same manner as bismuth oleate was a yellow solid.

Calculated for $C_{17}H_{31}CO_2Bi(OH)_2$: Bi, 40.0%; $C_{17}H_{31}CO_2$, 53.5%. Found: Bi, 39.7%; $C_{17}H_{31}CO_2$, 52.6%.

PREPARATION OF THE BISMUTH SALTS OF SATURATED FATTY ACIDS.

Bismuth Stearate.—This compound is a bulky, white solid which was prepared in substantially the same manner as the oleate. The product obtained here, however, was the tristearate and not a dibasic substance containing but one fatty acid radicle.

Calculated for $(C_{17}H_{35}CO_2)_3Bi$: Bi, 19.7%; $(C_{17}H_{35}CO_2)$, 80.3%. Found: Bi, 20.6%; $C_{17}H_{35}CO_2$, 74.4%.

Bismuth Palmitate.—This compound was prepared in the same manner as the oleate. It is a white powder.

Calculated for $(C_{15}H_{31}CO_2)_3Bi$: Bi, 21.46%; $C_{15}H_{31}CO_2$, 78.54%. Found: Bi, 19.47%; $C_{15}H_{31}CO_2$, 74.60%.

Bismuth Myristate.—This compound was prepared in the same manner as the oleate. It is a white solid.

Calculated for $(C_{13}H_{27}CO_2)_3Bi$: Bi, 23.38%; $C_{13}H_{27}CO_2$, 76.62%. Found: Bi, 25.47%; $C_{13}H_{27}CO_2$, 66.06%.

This compound was chiefly bismuth trimyristate containing some basic bismuth myristate.

Bismuth Laurate.—This was prepared in the same manner as the oleate. The white powder obtained was a mixture of monobasic and dibasic bismuth laurates as the following results show:

Calculated for $(C_{11}H_{23}CO_2)_2BiOH$; Bi, 33.47%; $C_{11}H_{23}CO_2$, 63.70%. Calculated for $C_{11}H_{23}CO_2Bi(OH)_2$: Bi, 47.3%; $C_{11}H_{23}CO_2$, 45.0%. Found: Bi, 36.78%; $C_{11}H_{23}CO_2$, 57.94%.

It is interesting to note that the unsaturated fatty acids yield chiefly dibasic bismuth salts and that the saturated fatty acids yield salts which are not basic until the length of the carbon chain and its molecular weight decreases. The fatty acids of lower molecular weight yield mixtures containing monobasic and dibasic salts.

THE PREPARATION OF BISMUTH SALTS OF DICARBOXYLIC FATTY ACIDS.

Bismuth Azelate.—Five Gm. of azelaic acid were dissolved in 25% alcohol and neutralized with alcoholic potash. While stirring, 35 cc. of a 16% solution of bismuth nitrate pentahydrate and 13% mannite were added. The white precipitate obtained was washed with water and dried. A monobasic bismuth salt was obtained.

Calculated for $C_7H_{14}(CO_2)_2BiOH$: Bi, 50.00%; $(CH_2)_7(CO_2)_2$, 45.1%. Found: Bi, 50.00%; $(CH_2)_7(CO_2)_2$, 42.3%.

Bismuth α-Octyl-α-Hydroxysebacate.—The same method was used as in the preparation of the oleate. The product was a white solid. The compound was a mixture of two or more bismuth salts.

Found: Bi, 42.2%; $(C_8H_{17})(OH)(C_8H_{16})(CO_2)_2$, 51.1%.

PREPARATION OF THE BISMUTH SALTS OF HYDROXY FATTY ACIDS.

Bismuth 9,10-Dihydroxystearate.—Ten Gm. of 9,10-dihydroxy stearic acid were dissolved in a hot mixture of 200 cc. of alcohol and 20 cc. of water; 1.27 Gm. of sodium hydroxide were added, and while stirring, 35 cc. of 16% bismuth nitrate pentahydrate and 13% mannite in water solution were added. The white precipitate which formed was washed with acetone to remove unreacted acid and dried. The product was a white solid. It was a mixture of the monobasic and dibasic salts according to the analysis.

Calculated for $(C_{18}H_{35}O_4)_2BiOH$: Bi, 23.96%; $C_{18}H_{35}O_2$, 72.18%. Calculated for $C_{18}H_{35}O_4Bi(OH)_2$: Bi, 37.4%; $C_{18}H_{35}O_4$, 56.4%. Found: Bi, 28.2%; $C_{18}H_{35}O_4$, 64.6%.

Bismuth Ricinoleate.—Twenty Gm. of ricinoleic acid were dissolved in alcoholic sodium hydroxide and the bismuth compound was precipitated by the addition of $^1/_3$ of an equivalent of the same bismuth nitrate solution used in other preparations. The yellow paste was washed with water and extracted with acetone. The final product obtained was a white solid, which on analysis was found to be a mixture of a monobasic bismuth salt and other bismuth salts.

Calculated for $C_{17}H_{33}OCO_2Bi(OH)_2$: Bi, 38.7%. Found: Bi, 44.38%.

PREPARATION OF BISMUTH SALTS OF FATTY ACIDS CONTAINING RING STRUCTURES.

Bismuth Chaulmoograte.—The chaulmoogric acid used was obtained from the saponification of chaulmoogra oil. This material was then recrystallized twice from 90% alcohol. The solid so obtained was contaminated by some hydnocarpic acid. Six Gm. of chaulmoogric acid were dissolved in 80% alcohol and treated with three equivalents of bismuth nitrate solution. The precipitate formed was washed with water and then extracted with acetone, leaving a solid which was chiefly the dibasic bismuth salt of chaulmoogric acid containing some bismuth hydnocarpate.

Calculated for $C_{17}H_{31}CO_2Bi(OH)_2$: Bi, 39.28%; $C_{17}H_{31}CO_2$, 52.45%. Found: Bi, 45.3%; $C_{17}H_{31}CO_2$, 45.5%.

Bismuth Hydnocarpate.—The hydnocarpic acid recovered from the purification of chaulmoogric acid was used in this preparation; 18 Gm. of acid were dissolved in 20% alcohol with the aid of alcoholic potash. The white solid bismuth compound was then precipitated by the addition of $^1/_3$ of an equivalent of bismuth nitrate solution. The white pasty precipitate obtained contained 19.53% of bismuth when dried.

The biological tests on these compounds were carried out in the Biological Laboratories of E. R. Squibb and Sons, New Brunswick, N. J.

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DETECTION OF DEXTROSE AND SUCROSE IN LACTOSE.

BY JOSEPH ROSIN AND F. C. HITCHCOCK.

The U. S. P. test for absence of dextrose and sucrose in Lactose is based on the greater solubility of the first two sugars in 70% alcohol, than of the latter. The test is as follows:

> Add 20 cc. of 70 per cent (by volume) alcohol to 2 Gm. of finely powdered lactose, shake the mixture frequently during half an hour at 15° C., and filter. A 10-cc. portion of the filtrate remains clear after mixing with an equal volume of dehydrated alcohol (dextrin), and this liquid upon evaporation on a water-bath leaves not more than 0.03 Gm. of residue (sucrose or glucose).

The 0.03 Gm. of residue permitted represents the quantity of lactose dissolved under the condition of the test. If dextrose or sucrose is present to any appreciable extent, the residue will be much greater.

Lactose was first introduced in the 1860 revision of the U. S. P. In the 1880 revision a charring test, with sulphuric acid, for sucrose was included. The alcohol solubility test for sucrose was first introduced in the 8th revision of the Pharmacopœia and has been continued in the subsequent revisions. The British, Netherlands, Swiss and some of the other Pharmacopœias also use, although with some modifications, the alcohol solubility in testing for sucrose. This test, up to a few years ago, worked very well. It is quite simple and, with one stroke, eliminates both sucrose and dextrose. Within recent years, however, objections have been voiced against this test. It is maintained that, owing to the application in the milk sugar industry, of modern manufacturing methods, such as spray drying, some amorphous lactose is produced. This form of lactose is more soluble in alcohol than the crystalline product and, consequently, yields more residue in the test. It has also been claimed, although it has not been proved yet, that some beta-lactose may be formed which has even a greater solubility in 70% alcohol than the amorphous variety. The amount of these varieties of lactose dissolved by 10 cc. of 70% alcohol are, approximately, of the following order:

Lactose, crystalline	0.03 Gm.
Lactose, dehydrated at 120° C	0.095 Gm.
Lactose, amorphous	0.105 Gm.
Beta-lactose, crystalline	0.125 Gm.

From this it is evident that the presence of even small quantities of the other forms of lactose would yield more residue in the test, and thus give an erroneous indication of the presence of dextrose or sucrose.

To meet this condition the producers of lactose recommended to the present Revision Committee of the Pharmacopœia that the test now official be replaced by tests specific for dextrose and sucrose.