Except in the case of Sample No. 2 the antimony trichloride test, as used by us, has given Vitamin A values which are in fair agreement with those obtained through biological assay.

We gratefully acknowledge the assistance of the Biological Laboratories of E. R. Squibb and Sons in conducting the biological assays reported herein.

ENTERIC COATINGS. II. EXCRETION STUDIES WITH SODIUM SALICYLATE TABLETS.*

BY MILTON WRUBLE.

In an earlier paper (1) the use of calcium sulphide-methylene blue tablets was found of value in checking the effectiveness of enteric coatings qualitatively. As a further step in this direction it was thought desirable to make a quantitative evaluation. Since salicylates are excreted more or less quantitatively, are widely used in medicine and in many cases produce irritation in the stomach when unprotected by a suitable coating, they were of particular interest in this connection.

Salicylates are excreted quite rapidly but incompletely in the urine, mainly as such, and to a small extent as salicyluric acid (2) and a number of other products. Stockman (3) states: "Salicylic acid and salicylates are conjugated and excreted as salicyluric acid. Persons taking up to 180 grains of sodium salicylate per day eliminate no free salicylic acid." Holmes has found (4) that for doses of sodium salicylate of from 2 to 5 Gm. the salicylic: salicyluric ratio is constant at the value of 40:60.

The absorption of salicylates is quite rapid and for this reason exceedingly small amounts are at times found in the feces, more often none at all. With full therapeutic doses of about 15 Gm., Hanzlik, Scott and Thoburn (5) and Hanzlik and Wetzel (6) were able to recover in the urine about 75 to 80 per cent of the total administered. They concluded that about 20 per cent of the salicylate was destroyed in its passage through the body.

The rate and duration of excretion of salicylates varies with the dosage, the individual and the individual's state of health. In general, it has been found by Blanchier (7) that with doses of 1 to 2 Gm., excretion is completed in 22 hours; Ehrmann (8) found that excretion lasted from 36 to 48 hours in normal individuals; Geissler (9) noted that complete elimination takes place in 12 hours. However, Sée (10) states that it ordinarily lasts from 24 to 48 hours.

The quantitative recovery of salicylates from tissues and body fluids involves difficulties and complexities not present in foods and simple aqueous solutions. This can be readily appreciated since in passing through the body the salicyl group is conjugated with glycocoll forming a salicyluric acid, whose properties differ from salicylic acid. Moreover, the presence of colloidal and other interfering substances prevent a smooth and quantitative recovery of salicyclic acid.

A number of quantitative methods for the determination of salicylates in urine have been developed. None appears entirely satisfactory. A critical survey of these methods has been adequately made by Thoburn and Hanzlik (11). Recently Merz (12) and Blume and Breuning (13) have outlined extraction methods for the determination of salicylates in urine.

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Since most of the proposed methods show decided weaknesses, Thoburn and Hanzlik have adapted a steam distillation method to the determination of salicylates in body fluids. Briefly their method (14) consists of:

1. Hydrolyzing an aliquot portion of urine which has been collected until the voided specimen, when extracted with ether and tested with ferric alum, is salicyl-free.
2. Distilling with steam until the salicylates are driven over.
3. Colorimetric estimation of the distillate with ferric alum.

Holmes has criticized (15) this method since he claims that the distillation of the salicylates is incomplete because the salicyluric acid is hydrolyzed only very slowly and that at the high temperatures which have to be used the salicyl ring is destroyed to some extent. He modifies the Thoburn-Hanzlik method in several details but his results indicate a lack of consistency.

Several of these methods were investigated by the author and the one finally adopted as most satisfactory was that of Thoburn and Hanzlik with several modifications. Holmes' method invariably yielded low results. The Merz method offered considerable difficulty because of the formation of troublesome emulsions and, as a result, the extraction process was long and tedious.

The Thoburn-Hanzlik method was modified in several details to yield better results. Considerable difficulty was experienced in completely recovering the salicylates from the average urine and with some urines the last traces were removed only after long and vigorous distillation. No doubt some urines are more highly conjugated than others and this accounts for the resistance displayed in the recovery of the salicylates. By keeping the volume of urine as low as possible during distillation, the recovery is considerably hastened. It was found advantageous, therefore, to begin with half the volume of urine recommended, namely, 50 cc.

Enough phosphoric acid is added to the urine to make it distinctly acid and the large excess recommended by Thoburn and Hanzlik avoided. Phosphoric acid proved to be the best hydrolyzing agent because of its high boiling point and also because it is not readily decomposed. However, with a large excess present it is quite possible that some is mechanically carried over into the distillate.

In this connection Thoburn and Hanzlik (16) make the following statement:

"The distillate should be perfectly clear, practically colorless and possess a nearly neutral or very slightly acid reaction to litmus paper."

However, Nicholls has shown that the solution should be appreciably acid. He states (17):

"Contrary to the usual statements in the literature, this test should not be applied to a neutral solution of a salicylate, as the color so produced is not of satisfactory shade. To obtain a good tint the solution should be slightly but appreciably acid, the intensity of color from a given quantity of salicylic acid decreasing with increasing acidity."

For the colorimetric comparison in Nessler tubes, salicylic acid rather than sodium salicylate as recommended, was used. It was found almost impossible to match the colors when sodium salicylate was employed as a standard. From what has already preceded it is apparent that the standard should be appreciably acid.

A 1 per cent solution of iron and ammonium sulphate which has been previously boiled and filtered was found to be superior to the 2 per cent solution

recommended by Thoburn and Hanzlik. The latter solution is considerably more intense in yellowish green color and with dilute solutions interferes with the colorimetric determination.

EXPERIMENTAL.

Eight individuals ranging in age from 18 to 25 years were each given three 5-grain tablets of sodium salicylate both uncoated and enteric coated.[1]

A complete 48-hour specimen of the urine was collected in each case. The urine samples were properly preserved and several determinations made on each sample. Distillation was carried out in each case until the ethereal extract of the residue showed no pink coloration with ferric alum.

The following table indicates the results obtained:

TABLE I.

Patient.	Total Volume Collected in 48 Hours.	Condition of Tablet.	Gm. Salicylate Recovered.
J. W. A.	1750 cc.	Coated	0.12 Gm.
	1905	Uncoated	0.18
E. J. V.	1100	Coated	0.33
	1570	Uncoated	0.39
E. E. B.	1635	Coated	0.38
	1550	Uncoated	0.24
M. F.	1875	Coated	0.38
	2675	Uncoated	0.35
K. K.	1450	Coated	0.14
	1325	Uncoated	0.23
D. F.	2370	Coated	0.53
	3380	Uncoated	0.36
W. W.	2225	Coated	0.30
	2210	Uncoated	0.30
S. K.	3620	Coated	0.28
	3280	Uncoated	0.25

CONCLUSIONS.

1. Several modifications of the Thoburn-Hanzlik method for the determination of salicylates in urine have been outlined.

2. The results indicate a close agreement between the quantities of salicylate excreted in the coated and uncoated tablets. Since no gastric irritation was reported, this is an indication of the effectiveness of the enteric coating.

3. The average recovery following the ingestion of 15 grains of salicylate appears to be approximately 30 per cent.

REFERENCES.

(1) Wruble, M., "Enteric Coatings," *Am. J. Pharm.*, 102, 318 (1930).

(2) Hanzlik, P. J., "Actions and Uses of the Salicylates and Cinchophen in Medicine," page 35.

(3) *Ibid.*, page 36.

(4) Holmes, E. G., "A Contribution to the Study of the Metabolism of Salicylic Acid," *J. Pharm. and Exp. Therap.*, 26, 313 (1925).

[1] This enteric coating was developed in the Research Laboratories of The Upjohn Company.

(5) Hanzlik, P. J., Scott, R. W., and Thoburn, T. W., "The Salicylates. V. Excretion of Salicyl in the Urines of Rheumatic and Non-Rheumatic Individuals," *J. Pharm. and Exp. Therap.*, 9, 247 (1917).

(6) Hanzlik, P. J., and Wetzel, N. C., "The Salicylates. XI. The Stability and Destruction of the Salicyl Group under Biological Conditions," *Ibid.*, 14, 25 (1919).

(7) Hanzlik, P. J., "Actions and Uses of the Salicylates and Cinchophen in Medicine," page 36.

(8) *Ibid.*, page 36. (9) *Ibid.*, page 36. (10) *Ibid.*, page 36.

(11) Thoburn, T. W., and Hanzlik, P. J., "The Salicylates. II. Methods for the Quantitative Recovery of Salicyl from Urine and Other Body Fluids," *J. Biol. Chem.*, 23, 163 (1915).

(12) Merz, K. W., "Ueber die Perkutane Resorption von Alkalisalizylaten aus Salbenaehnlichen Zubereitungen und die Quantitative Bestimmung der Salizylsaeure im Harn," *Archiv der Pharm.*, 269, 449 (1931).

(13) Blume, W., and Breuning, P., "Vergleichende Untersuchungen ueber den Resorptionsverlauf der Salizylsaeure nach intratrachealer und nach Subkutaner Injektion," *Archiv fuer Exp. Path. u. Pharm.*, 161, 183 (1931).

(14) Thoburn, T. W., and Hanzlik, P. J., "The Salicylates. II. Methods for the Quantitative Recovery of Salicyl from Urine and Other Body Fluids," *J. Biol. Chem.*, 23, 174 (1915).

(15) *J. Pharm. and Exp. Therap.*, 26, 297 (1925).

(16) *J. Biol. Chem.*, 23, 175 (1915).

(17) Nicholls, J. R., "Determination of Small Quantities of Benzoic and Cinnamic Acids with Some Notes on the Colorimetric Determination of Salicylic Acid," *Analyst*, 53, 19 (1928).

(*To be continued.*)

COMPOUND SOLUTION OF CRESOL—THE VARIATION OF PHENOL COEFFICIENT WHEN DIFFERENT OILS ARE USED FOR SAPONACEOUS BASE.*,[1]

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The Bureau of Animal Industry of the United States Department of Agriculture has been very active in its supervision of the interstate shipment of domestic animals, and as a part of their duties they have described means for disinfecting cages as well as animals. Since such a procedure is common and within their control, it is only a natural sequence that they should be highly interested in the control of the material used in these prescribed methods of disinfecting. This department deviated from the standards which were laid down by the United States Pharmacopœia for compound solution of cresol as early as 1915 (1). They deemed it necessary, in view of their extensive recommendations for the use of soap solutions of cresol, to lay down requirements in many cases more stringent than those in the United States Pharmacopœia. The economy of manufacture was given due consideration when making these specifications as well as the effectiveness of the final product.

In the interest of improving compound solution of cresol U. S. P. the authors tried several oils which are available for use in the manufacture of such a product. Compound solutions of cresol were prepared, following the directions of the United States Pharmacopœia, tenth revision, using corn oil, peanut oil, sesame oil, cocoanut oil, and soy bean oil (2). In order to have a control sample, a solution was made

* Scientific Section, A. PH. A., Portland meeting, 1935.

[1] From the Control Laboratories, Eli Lilly and Company.