ical loss rather than to the procedure. Since this was the first compound made, unlabeled tropine HCl, sufficient to increase the total weight to the 124-mg. weight normally obtained, was added to the tropine-2,3,4-^{14}C HCl. For this reason the specific activity of the product was correspondingly decreased to 0.62 mc./mmole. In subsequent experiments, the labeled compounds were not diluted.

The radiochemical purity of the resulting labeled atropines was established by paper and thin-layer chromatography as described previously (8). Each of the labeled atropines contained an impurity that chromatographed in all systems as tropine. The impurity, varying from 2 to 6% of total radioactivity (depending on labeled product), could be removed by a second recrystallization from petroleum ether. The yields reported in Table I have been corrected for the impurity and accurately reflect the yields of the products shown.

SUMMARY AND CONCLUSIONS

Using the microsynthetic procedures described previously (7, 9), three different tropine-labeled atropines were prepared. From citric-3-^{14}C acid, tropanone-3-^{14}C (44%), and atropine-3-^{14}C (26%) were synthesized with a specific activity of 1 mc./mmole. From citric-2,4-^{14}C acid was prepared tropanone (68%), tropine (65%), and atropine (34%) having specific activity of 1 mc./mmole and correspondingly labeled. From citric-2,3,4-^{14}C acid was prepared correspondingly tropanone (54%), tropine (53%), and atropine (25%).

The labeled atropine contains a tropine impurity that varied from 2% to 6% of the total radioactivity. This impurity could be removed by recrystallization from petroleum ether.

This report described the first synthesis, by other than biosynthetic method, of tropine and atropine selectively labeled with ^{14}C in the tropine ring.

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Keyphrases

Tropine-labeled atropine—synthesis
Atropine—citric-^{14}C acid labeling of 2, 3, 4 positions
Paper chromatography—analysis, radiochemical purity
TLC—analysis, radiochemical purity

Correlation Between *In Vitro* and *In Vivo* Disintegration Times of Enteric-Coated Tablets

By SØREN RASMUSSEN

Investigations were made with enteric-coated tablets on the correlation between the first appearance of a substance in the plasma or the urine and the thickness of the vernix layer, measured by the *in vitro* disintegration time. The content of the tablets is quinine hydrochloride, sodium sulfanilate, or sodium *p*-aminosalicylate. The content of each tablet was either 50 or 500 mg. It was shown that within preparations of the same content a proportionality exists between the *in vivo* and the *in vitro* disintegration times. Between series of preparations with different content the factor of proportionality may differ significantly.

NUMEROUS PAPERS have been published on the correlation between *in vitro* tests and the therapeutic effectiveness of pharmaceutical preparations. The principal aim of such investigations is to establish a predictability of biological properties from simple *in vitro* tests.

With oral preparations of different types, much important work has been done by Levy and his collaborators (1). As to the dissolution rate, it was possible in some cases to show a correlation between the results of *in vitro* and *in vivo* determinations. In such cases it should be possible to predict from *in vitro* tests

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variations in the *in vivo* dissolution rate from batch to batch.

However, with enteric-coated tablets a further problem arises. The time from the intake until the drug appears in the plasma, which is an indication of the beginning of the absorption, may vary independently of the dissolution rate and must be determined separately. Many *in vitro* tests for this disintegration time exist, but the correlation between the *in vitro* and the *in vivo* disintegration time is little investigated.

In some cases a certain connection is found, as appears from Morrison and Campbell (2). Most of the investigation has been done with commercial preparations, and information about the nature and the amount of vernix is lacking. More precise information is given by Wagner *et al.* (3–5) in works with $BaSO_4$-containing tablets coated with increasing amounts of vernix. They followed the *in vivo* disintegration roentgenographically and were able to show a correlation between the *in vivo* and the *in vitro* disintegration times. In a previous paper Rasmussen (6) showed an increase in the *in vivo* disintegration time of enteric-coated tablets of quinine hydrochloride of 50 or 500 mg. proportional to the thickness of the coating layer.

In a subsequent paper Rasmussen (7) demonstrated a rectilinear proportionality between the thickness of the vernix layer and the disintegration time *in vitro*. Measured on tablets of quinine hydrochloride, sodium sulfanilate, or sodium *p*-aminosalicylate of 50 or 500 mg. each, the same factor of proportionality was found, independent of size and content. It was still an open question if a similar constant factor of proportionality could be found between the thickness of the vernix layer and the disintegration time *in vivo*.

This paper reports investigations on the *in vivo* disintegration of tablets of quinine hydrochloride, sodium sulfanilate, or sodium *p*-aminosalicylate of various sizes and compositions and coated with various amounts of vernix.

As will be seen, it was not possible to show a proportionality factor which is independent of the content of the tablets.

EXPERIMENTAL

Preparations

The coated preparations are manufactured from tablets of the following compositions.

Tablets of Quinine Hydrochloride—Preparation No. 6–15: the composition appears from an earlier paper (6). The tablets contain either 50 or 500 mg. of quinine hydrochloride. Preparation No. 46–50: for each tablet take 50 mg. of quinine hydrochloride, 45 mg. of granulatum simplex (8), and 5 mg. magnesium stearate. Granulate by compression, mix, and compress. Spherical punches, 6 mm. diameter.

Tablets of Sodium Sulfanilate—For each tablet take 50 mg. of sodium sulfanilate and 40 mg. of starch. Mix and prepare by moist granulation. To the dried granulate add 10 mg. of talcum. Mix and compress. Spherical punches, 6 mm. diameter.

Tablets of Sodium *p*-Aminosalicylate—For each tablet take 50 mg. of sodium *p*-aminosalicylate, 45 mg. of granulatum simplex (8), 4 mg. of talcum, and 1 mg. of magnesium stearate. Mix and prepare by compression. Spherical punches, 6 mm.

The coating is made with vernix enterosolubilis (9): 10 mg. of cellulose acetate phthalate, 500 mg. of castor oil, and 89.5 g. of acetone. The vernix was applied to the tablets in an open pan. From time to time during the coating, numbers of 500–1,000 tablets were removed from the pan and dried, whereas the rest was coated further. This procedure ensures that subbatches removed during the coating only differ in the amount of vernix applied.

In Vitro Experiments—The alkaline disintegration time was determined by the following modification of the method used by the Nordic Pharmacopoea (10). Place three tablets in a conical flask in a solution of 0.285 g. of sodium phosphate, and 0.055 g. of potassium phosphate monobasic to 30 ml. of water maintained at 40°. Shake the flask gently during the whole of the disintegration. The point of disintegration is defined as the moment when the coating breaks up.

The acidic pretreatment is made as follows: Place the tablets in a mixture of 3 ml. phosphoric acid 1 *M* and 27 ml. water. Shake every 5 min. for 2 hr., pour off, and wash with 5 ml. water.

In Vivo Experiments—Eighteen healthy volunteers served as experimental subjects. The tests were started between 8:30 and 9:00 a.m., 1–1.5 hr. after a light breakfast. The test dose of 0.5 g. was ingested along with 150–200 ml. of water. Blood from the earlobe or urine was collected immediately before and every hour after the ingestion. The difference in time between the ingestion and the first appearance of the substance in the plasma or the urine was defined as the *in vivo* disintegration time. As the samples were collected every hour, the disintegration time was measured in whole hours.

Analytical

Quinine was determined in 0.1 ml. heparinized plasma by the method of Brodie and Udenfriend (11). Sodium *p*-aminosalicylate was determined in the plasma by the following method: 0.10 ml. heparinized plasma was mixed with 1 ml. water. After 15 min. the proteins were precipitated by adding 0.25 ml. 10% trichloroacetic acid. After 20 min. the tubes were centrifuged at $1100 \times g$ for 15 min.; 1 ml. of the clear supernatant was transferred to a tube containing 1.20 ml. of the following solution: 145 ml. of sodium hydroxide 1 *N*; 8.58 g. of sodium borate; to 1000 ml. of water, which gives pH = 11.

The fluorescence was read on an Aminco-Bowman

TABLE I—ALKALINE *In Vitro* DISINTEGRATION TIMES OF ENTERIC-COATED TABLETS WITH AND WITHOUT PRETREATMENT IN ACID FOR 2 HR. AND *In Vitro* DISSOLUTION RATES OF SOME OF THE PREPARATIONS

Content	Preparations No.	Disintegration Time, min. With Pretreatment $\bar{x} + SEM$	(n)	Disintegration Time, min. Without Pretreatment $\bar{x} \pm SEM$	(n)	% Disintegration During the 2 Hr. in the Acid	*In Vitro* Dissolution Half-lives, min.
		Uncoated Tablets					
Quinine hydrochloride	5	7.1 ± 1.3	(26)	1.4 ± 0.3	(30)[a]	13	26.4
	47	2.7 ± 0.4	(55)	1.9 ± 0.4	(30)[a]	8	
	12	**100% Disintegration in the Acid**					
	48	2.66 ± 0.06	(60)	3.25 ± 0.09	(30)[a]	0	
	7	4.5 ± 0.2	(20)	3.97 ± 0.09	(30)	0	31.3
	13	4.7 ± 0.6	(21)	4.6 ± 0.2	(30)	30	
	49	6.36 ± 0.12	(60)	6.20 ± 0.14	(30)	0	51.0
	15	7.41 ± 0.19	(30)	8.1 ± 0.2	(20)	0	53.8
	50	13.2 ± 0.2	(60)	12.4 ± 0.2	(30)	0	56.8
	14	20.9 ± 1.1	(30)	22.5 ± 0.9	(30)	0	
Sodium sulfanilate	22	**Uncoated Tablets**					1.2
	23	2.10 ± 0.14	(20)	2.03 ± 0.07	(30)	0	1.7
	24	2.9 ± 0.2	(20)	3.75 ± 0.12	(30)	0	1.6
	25	6.2 ± 0.3	(20)	6.53 ± 0.13	(30)	0	2.8
Sodium *p*-aminosalicylate	41	**100% Disintegration in the Acid**					7.8
	42	6.26 ± 0.06	(27)	7.8 ± 0.2	(30)[a]	10	14.6
	43	12.5 ± 0.5	(30)	13.5 ± 0.4	(30)	0	11.9

[a] Denotes $p < 0.01$.

spectrofluorometer at 400 mμ, activated at 310 mμ (both uncorrected). Standards and blank were run through the procedure. No differences were found between standards made in plasma and water, and generally aqueous solutions were used. Concentrations down to 0.1 mcg./ml. are detectable with a recovery of 99–100%. The specificity of the method with respect to metabolites has not been investigated, but the appearance of a fluorescence in the plasma is taken as indication of an absorption of sodium *p*-aminosalicylate. Sodium sulfanilate was determined in the urine by the method of Morris (12).

RESULTS

Table I presents the alkaline *in vitro* disintegration times of the coated preparations with and without acid pretreatment.

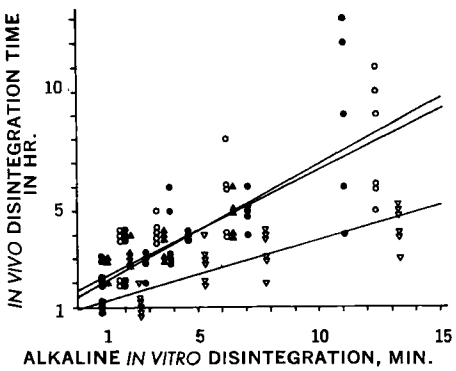

Fig. 1—In vivo *disintegration times of enteric-coated tablets* versus *alkaline* in vitro *disintegration times. Key:* ○●, *quinine HCl preparations;* ▽, *Na-p-aminosalicylate preparations;* ▲, *Na-sulfanilate preparations. The lines are drawn from the equations of regression.*

The *in vivo* disintegration times from all experiments are plotted in Fig. 1 against the means of the *in vitro* disintegration times from Table I. No difference was found between the results with the 50-mg. and the 500-mg. tablets of the preparations No. 6–15, and these preparations are treated as one group.

Regression lines were calculated from the determinations as pairs of numbers. Three instances in which no *in vivo* disintegration was detected are omitted. Tests with uncoated tablets, where the disintegration times are: *in vitro* 0 min., *in vivo* the first hour, are included in the calculations.

The equations of regression are found as follows:

$$Y_Q^{(1)} = 0.60X + 1.14 \quad r = 0.82 \quad n = 40$$
$$Y_Q^{(2)} = 0.50X + 1.96 \quad r = 0.84 \quad n = 26$$
$$Y_Q = 0.56X + 1.47 \quad r = 0.82 \quad n = 66$$
$$b/s_b = 11.6$$
$$Y_{PAS} = 0.26X + 0.98 \quad r = 0.88 \quad n = 22$$
$$b/s_b = 3.18$$
$$Y_{SUL} = 0.50X + 1.70 \quad r = 0.87 \quad n = 30$$
$$b/s_b = 11.6$$

Y is the *in vivo* disintegration in hours of tablets of quinine hydrochloride (Q), sodium *p*-aminosalicylate (PAS), and sodium sulfanilate (SUL), respectively, and X is the *in vitro* disintegration in min.

$Y_Q^{(1)}$ is calculated from tests with the preparations No. 6–15, manufactured differently from the preparations No. 46–50, from which $Y_Q^{(2)}$ is calculated. The two regression coefficients do not differ significantly from each other ($t = 1.00$, $p > 0.3$, $df = 62$), and neither do the positions of the lines ($t = 0.923$, $p > 0.3$, $df = 62$). This permits the calculation of an equation of regression Y_Q, based on all tests with the quinine preparations. The ratios b/s_b show that all regression coefficients differ significantly from zero ($p < 0.005$). The intercepts of the regression lines with the ordinate, do not differ significantly from the expected point (0.1), *i.e.*, the disintegration times of uncoated

tablets in tests with sodium *p*-aminosalicylate and quinine HCl: $t_Q = 1.6$, $p > 0.1$, $df = 64$ and $t_{PAS} = 0.71$, $p > 0.4$, $df = 28$, whereas the difference is significant with sodium sulfanilate: $t_{SUL} = 2.92$, $p < 0.01$, $df = 20$. The comparison between the regression coefficients shows: b_Q/b_{PAS}, $t = 5.25$, $p < 0.001$, $df = 28$; b_{SUL}/b_{PAS}, $t = 3.41$, $p < 0.005$, $df = 20$; $b_Q/_{SUL}$, $t = 0.61$, $p > 0.5$, $df = 84$.

DISCUSSION

The results are in agreement with those of Wagner *et al.* (3–5), who found correlation between the *in vitro* and the *in vivo* disintegration times, but a further important point has to be added. The statistical analysis of the results presented here shows that the lines fitted to tests with quinine HCl and sodium sulfanilate differ significantly from the line fitted to the tests with sodium *p*-aminosalicylate. This means that the factor of proportionality by which the *in vivo* disintegration time increases with increasing thickness of the coating layer may differ significantly with different contents of the tablets. It is thus found that tablets showing an *in vitro* disintegration time of 12 min. take about 8 hr. to disintegrate *in vivo* when the content is quinine or sulfanilate, and about 4 hr. when the content is *p*-aminosalicylate.

The *in vivo* disintegration time, *i.e.*, the time until analytically sufficient concentrations are built up in the plasma or the urine, may be influenced by the rate of dissolution from the preparations in the way that a decreased dissolution rate may increase the time between the *in vivo* break-up of the coating layer and the moment when detectable concentrations are reached, and in this way apparently increase the *in vivo* disintegration time.

If this explanation were valid, one should expect that sodium *p*-aminosalicylate were released at a higher rate than are quinine hydrochloride and sodium sulfanilate from the coated preparations in order to explain the differences found.

However, *in vitro* dissolution half-lives of some of the coated preparations, listed in Table I, show that sodium sulfanilate is released at a much higher rate than sodium *p*-aminosalicylate or quinine hydrochloride, although the former is released faster than the latter.

Another way in which the *in vivo* disintegration time may be altered is by some influence of the acid in the stomach. The influence is tested by determinations of the alkaline *in vitro* disintegration time with and without acid pretreatment. Table I shows that significant alterations are only found with preparations with such a thin vernix layer that some of the tablets disintegrate during the 2 hr. in the acid.

The delay in the *in vivo* disintegration time of the coated preparations could further be explained as a retention in the stomach. Nelson (13) calculated an average stomach retention time of 3 hr., from the results of Blythe *et al.* (14) who worked with coated $BaSO_4$ tablets. If so, the regression lines would intercept the ordinate at a point near to 3 hr., *i.e.*, at a point corresponding to the retention time. This is not the case with any of the preparations in this work. Only the line calculated from tests with sodium sulfanilate tablets has an intercept significantly different from the expected point: (0,1). This, on the other hand, may be explained by the fact that the disintegration times here are determined by the appearance of sulfanilate in the urine, where the limit of detection is not very low because of the variations in the blank readings.

CONCLUSION

Batches of enteric-coated tablets of the same substance show an increase in the *in vivo* disintegration time proportional to the increase in the *in vitro* disintegration time, independently of the size and pharmaceutical composition of the tablets.

Between preparations of different substances the factor of proportionality may differ significantly.

As a consequence, the predictability of the *in vivo* fate of the drug requires determination of the correlation between *in vivo* and *in vitro* dissolution rates, as well as the factor by which the *in vivo* disintegration time changes with the *in vitro* disintegration time. This factor of proportionality has to be evaluated for each substance.

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Keyphrases

Disintegration time—enteric-coated tablets
In vivo, *in vitro* disintegration time—correlation
Tablet formulation effect—disintegration time
Enteric coating thickness—disintegration time
Fluorometry—analysis, plasma