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Transformation and Excretion of Drugs in Biological Systems. IV.[1] Reabsorption of Biliary Metoclopramide-N^4-glucuronide and -N^4-sulfonate from Rabbit Intestine[2]

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Of metoclopramide-N^4-glucuronide and -N^4-sulfonate, both of which were found as the major constituents in the bile after metoclopramide administration to rabbits, reabsorption from the intestine was studied to explore the possibility of the entero-hepatic circulation. Following the administration of the N^4-conjugates into the rabbit duodenum, the urinary constituents were quantitatively measured at various times over a period of 12 hours. In the case of the sulfonate, all recovered in the urine was found to be the sulfonate. The result suggests that this conjugate itself may be absorbed from the intestine without being hydrolysed. On the other hand, when the glucuronide was intraduodenally given, at least two features of urinary excretion were observed: One showed that the glucuronide accounts for more than 90% of the total recovered in 8 hours, and the other did that the proportion of it to the total decreases with time. From the examinations for the stability in the aqueous solution and for the urinary excretion after the intravenous administration, it was concluded that the glucuronide may be absorbed from the intestine not only in the parent compound, namely, metoclopramide resulting from the hydrolysis but in the intact form as well as the sulfonate.

In the previous paper,[1] in the bile of rabbits receiving an intravenous dose of metoclopramide, the unchanged drug and its N^4-conjugates, namely, the glucuronide and the sulfonate being the major constituents similar to those observed in the urine[4] were separately determined, and it was found that the two N^4-conjugates account for a large part of the total recovered.

The biliary excretion of drugs in connection with the enterohepatic circulation is important as one of the factors affecting the duration of drug action. From such a view-point a number of foreign compounds, especially long acting cardiac glycosides, have been studied.[5] When a compound is excreted into the intestine *via* the bile, it is said to be largely in the form of conjugates such as glucuronides, glycine conjugates, ethereal sulfates, and so on;[6] most of them are polar acidic compounds which may not be readily absorbed from the gut. It is, therefore, interesting to know what is the fate of these conjugates in the intestine. Most of the workers[7] who studied of this problem have suggested that these conjugates could be hydrolysed by enzymes present in the gut secretions or in gut bacteria and resulting original compounds could be absorbed into the blood.

Hitherto, however, little or nothing has been done to investigate the fate of N-conjugates of aromatic amines in the intestine. Such being the case, using the N^4-conjugates of meto-

1) Part III: T. Arita, R. Hori, K. Ito, and K. Ichikawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1670 (1970).
2) This work was presented at the 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1969.
3) Location: *Kita-12, Nishi-6, Sapporo.*
4) T. Arita, R. Hori, K. Ito, K. Ichikawa, and T. Uesugi, *Chem. Pharm. Bull.* (Tokyo), **18**, 1663 (1970).
5) H.J. Kupferberg and L.S. Schankl, *Am. J. Physiol.*, **214**, 1048 (1968); G.T. Okita, *Federation Proc.*, **26**, 1125 (1967); *etc.*
6) R.T. Williams, P. Millburn, and R.L. Smith, *Ann. N. Y. Acad. Sci.*, **123**, 110 (1965).
7) H.B. Hucker, A.G. Zacchei, S.V. Cox, D.A. Brodie, and N.H.R. Cantwell, *J. Pharmacol. Exptl. Therap.*, **153**, 237 (1966); G. Levy, L. Weintraub, T. Matsuzawa, and S.R. Oles, *J. Pharm. Sci.*, **55**, 1319 (1966); A.J. Glazko, W.A. Dill, and L.M. Wolf, *J. Pharmacol. Exptl. Therap.*, **104**, 452 (1952); *etc.*

clopramide as an interesting example, the absorption behavior in rabbits is studied by the method of intraduodenal administration.

Experimental

Animals——Male albino rabbits weighing 2.5 to 3.0 kg were used.

Materials——Metoclopramide and its N^4-glucuronide and N^4-sulfonate used were prepared as described previously.[4]

Preparation of Sample Solution——Metoclopramide was dissolved in 20 ml of 0.9% NaCl by adjusting to pH 4.5 with 1N HCl. Both the N^4-glucuronide and the N^4-sulfonate were dissolved in 20 ml of 0.9% NaCl.

Method of Intraduodenal Administration——Rabbits were fasted for about 30 hr prior to the experiments but were allowed free access to water. The animals were anesthetized by the intravenous injection of pentobarbital sodium (25 mg/kg body wt.). The upper part of the small intestine was exposed by a midline abdominal incision and the sample solution was injected through a syringe near an opening of biliary duct in the duodenum lumen. The intestine was replaced in the abdomen, the incision was closed. Urine collections were made at various times during the experiment through Nelaton's catheter inserted to the bladder, and the samples (pH 6—8) were analysed immediately.

Method of Intravenous Administration——Ten milliliters of 0.9% NaCl containing the glucuronide was given intravenously to rabbits. Urine and bile collections were made by the method similar to those described previously.[1]

Analytical Method——Each of metoclopramide and its N^4-conjugates in the biological samples collected was separately determined by the previous method.[1]

Examination for Hydrolysis of Metoclopramide-N^4-glucuronide——Each buffer for the hydrolysis reaction was made by HCl–sodium acetate (pH 1—5) and M/15 KH_2PO_4–M/15 Na_2HPO_4 (pH 5—8), and the ionic strength was adjusted with NaCl (see Fig. 5 and Table I). After adding 5 ml of freshly prepared water solution of the glucuronide (10 mg/ml) to 45 ml of the buffer under the condition of $37.0 \pm 0.1°$, at various times 1 ml of the reaction mixture was pipetted into a test tube containing 9 ml of 0.1N NaOH to stop the reaction. Isolation and determination of resulting metoclopramide were carried out by the procedure described previously.[1]

Result and Discussion

Following a 100 mg intraduodenal dose of metoclopramide, the urinary excretion of the unchanged drug and its N^4-glucuronide and N^4-sulfonate with time is shown in Fig. 1. In this case, the decreasing order of amount of the recovered excrements exhibited a tendency similar to that obtained with the intravenous administration[1] as follows: N^4-glucuronide>N^4-

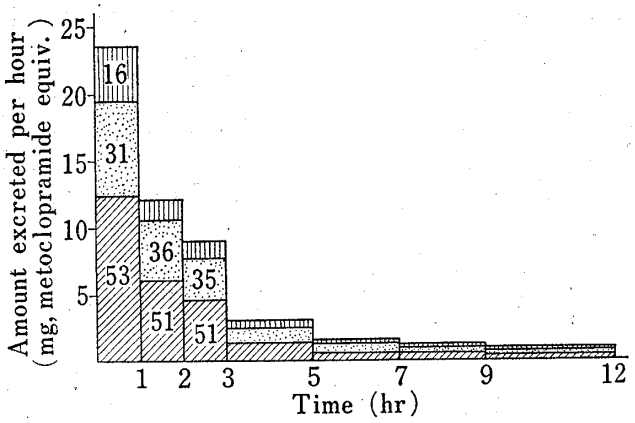

Fig. 1. Urinary Excretion of Metoclopramide and Its N^4-Conjugates after Intraduodenal Administration of Metoclopramide to Rabbit (Dose: 100 mg; Total Amount recovered in 12 hr: 54.6% of the Dose)

▥: metoclopramide, ▦: metoclopramide-N^4-sulfonate, ▨: metoclopramide-N^4-glucuronide
Numbers in rectangles indicate the percentages of each excrement to total amount recovered in each time interval.

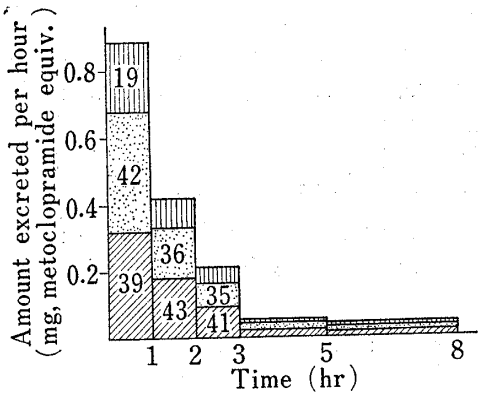

Fig. 2. Urinary Excretion of Metoclopramide and Its N^4-Conjugates after Intraduodenal Administration of Metoclopramide to Rabbit (Dose: 6 mg; Total Amount recovered in 8 hr: 30.0% of the Dose)

See explanation for bar graph of Fig. 1.

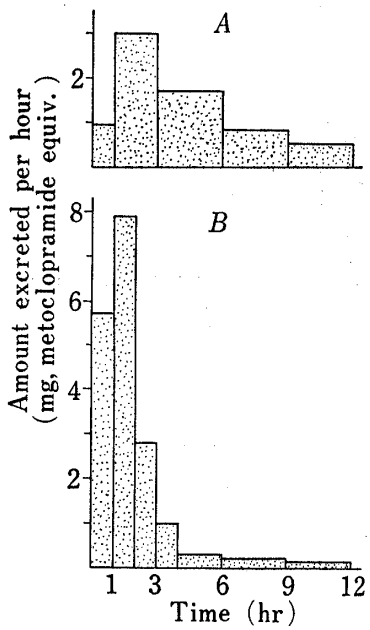

Fig. 3. Urinary Excretion of Metoclopramide and Its N^4-Conjugates after Intraduodenal Administration of Metoclopramide-N^4-sulfonate to Rabbit (Dose: 130 mg equiv. of Metoclopramide; Total Amount recovered in 12 hr: 12.4% (*A*) and 15.4% (*B*) of the Dose; In this Case, no Presence of Metoclopramide and Its N^4-Glucuronide was observed)

See explanation for bar graph of Fig. 1.

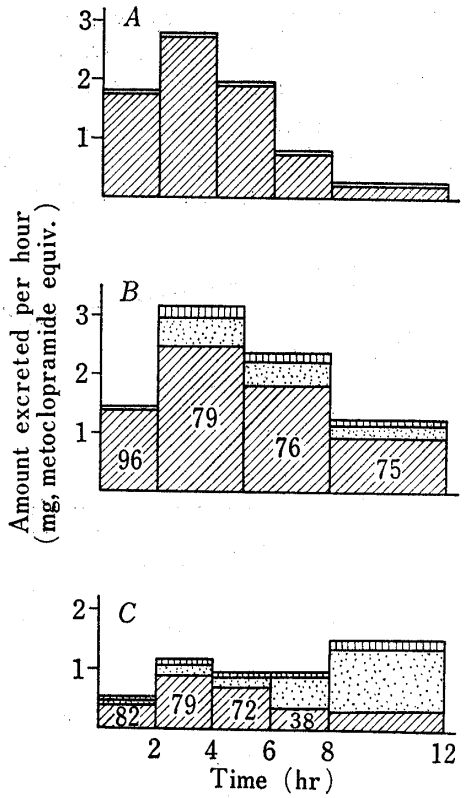

Fig. 4. Urinary Excretion of Metoclopramide and Its N^4-Conjugates after Intraduodenal Administration of Metoclopramide-N^4-glucuronide to Rabbit (Dose: 95 mg equiv. (*A* and *C*) and 190 mg equiv. (*B*) of Metoclopramide; Total Amount recovered in 12 hr: 16.4% (*A*), 12.7% (*B*), and 14.9% (*C*) of the Dose)

See explanation for bar graph of Fig. 1.

sulfonate>unchanged metoclopramide; difference of which, however, was never large in comparison with the case of the administration of the glucuronide, except for Fig. 4C, or of the sulfonate as will be mentioned elsewhere in this paper. A maximum of hourly excretion was observed within one hour after the administration, and 54.6% of the dose was recovered in 12 hours.

In order to examine for the influence of doses on a quantitative pattern of the three excrements, a six mg dose being considerably small was given to the rabbit. As shown in Fig. 2, the result gives a similar tendency to that in Fig. 1.

The intraduodenal administration of metoclopramide-N^4-sulfonate gave the urinary excretion pattern as shown in Fig. 3; in which it is interesting to note that a maximum of hourly excretion was observed at somewhat later time than that in the case of metoclopramide administration. In addition, that the total amout recovered in 12 hours was wholly of the sulfonate suggests that this componud may be absorbed from the intestine in the intact form without being hydrolysed. No hydrolysis of the sulfonate was observed for at least 48 hours at 37° in various pH solutions (pH 4—13).

On the other hand, when metoclopramide-N^4-glucuronide was intraduodenally given to rabbits, the features of urinary excretion were not simple as seen in Fig. 4 A—C. Fig. 4A shows that in the urine of each collection period by 8 hours the amount of the glucuronide is more than 90% of the total recovered. Fig. 4B, moreover, shows that the greater part

of the total urinary recovery, especially at the earliest period (0—2 hour), was found as the glucuronide, though the other two excrements also were present to some extent. It is thus expected that the glucuronide also may be absorbed from the intestine in the intact form. This finding, together with that for the above sulfonate, may perhaps be used for a better understanding of absorption behavior of such polar compounds being highly water-soluble and not necessarily small in molecular size.

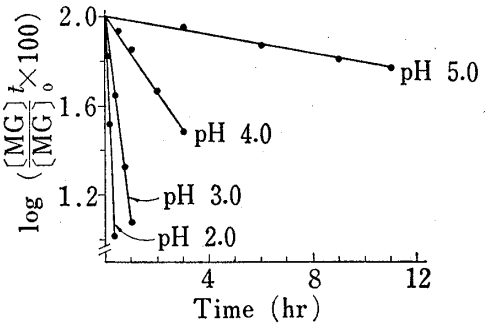

Fig. 5. Hydrolysis of Metoclopramide-N⁴-glucuronide in Various pH Solutions at 37° and Ionic Strength 0.2

$[MG]_0$: initial concentration of the glucuronide
$[MG]_t$: concentration of the glucuronide at time t

pH	1M sodium acetate, ml	1N HCl, ml	NaCl, g	
2.0	50.0	52.5	0	added water to make up to 500 ml
3.0	50.0	48.5	0.234	
4.0	50.0	40.0	0.730	
5.0	50.0	15.0	2.190	

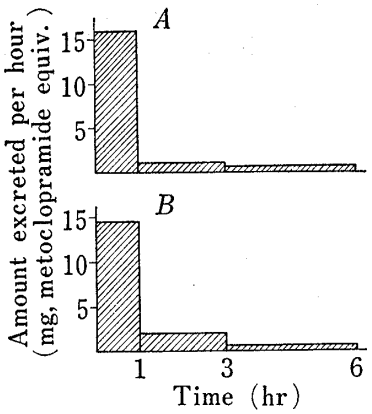

Fig. 6. Urinary Excretion of Metoclopramide and Its N⁴-Conjugates after Intravenous Administration of Metoclopramide-N⁴-glucuronide to Rabbit (Dose: 25 mg equiv of Metoclopramide; Total Amount Recovered in 6 hr: 75.8% (*A*) and 78.2% (*B*) of the Dose; In this Case, no Presence of Metoclopramide and Its N⁴-Sulfonate was Observed)

See explanation for bar graph of Fig. 1.

In the case of Fig. 4C, a peak appeared in 2—4 hour period like the above two cases of the glucuronide administration; however, the proportion of the glucuronide to the total recovered decreases with time. It may be inferred from these that in the lower part of the intestinal tract the glucuronide is probably hydrolysed by some causes to metoclopramide which is more absorbable.

As is evidently seen in Fig. 5, the hydrolysis reaction of metoclopramide-N⁴-glucuronide (MG) in aqueous solution, MG+H_2O→metoclopramide+glucuronic acid, is first order and the rate depends on the pH. The reaction rate constants and the half-lives are given in Table

Table I. Rate Constants k and Half- lives $t_{1/2}$ for the Hydrolysis of Metoclopramide-N⁴-glucuronide in Various pH Solutions at 37° and Ionic Strength 0.2

pH	k, hr^{-1}	$t_{1/2}$, hr
2.0	6.42	0.108
3.0	2.10	0.335
4.0	0.384	1.80
5.0	0.0449	13.9
6.0[a)]	0.00720	96.2
6.8[a)]	0.00185	375

a) M/15 KH_2PO_4+M/15 Na_2HPO_4+NaCl=90 ml+10 ml+0.729 g (pH 6.0), 40 ml+60 ml+0.338 g (pH 6.8)
See Fig. 5 concerning the composition of each solution of pH 2—5.

Table II. Influence of Ionic Strength μ on the Hydrolysis of Metoclopramide-N^4-glucuronide in Solutions of pH 2 and 3 at 37°

μ	Rate constant, hr^{-1}	
	pH 2	pH 3
0.2	6.42	2.10
0.5[a)]	7.98	2.46
1.0[a)]	9.66	3.32

a) Prepared by adding further NaCl to the corresponding buffer given in Fig. 5.

I. At the acid pH the glucuronide is markedly unstable; but the higher the pH, the greater the stability becomes, and in the aqueous solution of pH 8—13 no hydrolysis was yet observed at one week after the start of the experiment. While, the influence of ionic strength on the hydrolysis was examined at pH 2 and 3. From Table II it is understood that the rate increases with the ionic strength.

The pH of the intestinal fluid is said to range usually from 6 to 8.[8)] From the rate constant shown in Table I, it can be estimated that, under the experimental condition of pH 6, only about 7% of the initial glucuronide concentration is hydrolysed for the first 10 hours. In the intestinal tract, accordingly, the glucuronide is thought to be considerably stable. However, it must be taken into account that some exogenous or endogenous factor brings about a change in pH and/or ionic strength of intestinal fluid to be more favorable for the hydrolysis of the glucuronide and that this N-glucuronide might be hydrolysed by enzymes present in the intestinal tract as has been pointed out concerning some ethereal and ester glucuronides.[6)] It is perhaps important as a factor to note that the rabbit given an abnormal pattern of urinary excretion (Fig. 4C) had kept mashy remains throughout the small-intestinal tract because of the insufficiency of fasting.

Thus, the three cases of the glucuronide dose are thought to have exhibited its own feature. Considering that no such observation was made in the case of the sulfonate being much more stable over the pH range examined, this complex phenomenon, especially in Fig. 4C, are suspected to be due mainly to a change in the chemical conditions of environment.

In addition, the glucuronide might be hydrolysed in body fluid other than the intestine after it was absorbed and entered the blood stream. In order to approach a solution of the problem, the urinary excretion of the rabbit receiving the glucuronide intravenously was examined. In this case, as is seen in Fig. 6, about 80% of the dose was recovered in 6 hours after the administration, and all of which was found as the glucuronide. In the bile specimens also collected simultaneously, only the glucuronide was recovered. Accordingly, it seems probable that under the conditions of the experiments the hydrolysis of this N-glucuronide to the parent compound occurs in the intestine, but not in the other body fluid.

Thus, it may be concluded that metoclopramide-N^4-glucuronide excreted in the bile is reabsorbed from the intestine not only in the parent compound resulting from the hydrolysis but in the intact form as well as metoclopramide-N^4-sulfonate.

Acknowledgement Thanks are given to Miss M. Aizawa for her assistance in the experimental work.

8) T.H. Wilson, "Intestinal Absorption," W.B. Saunders Company, Philadelphia, 1962, pp. 247—250; G. Wiseman, "Absorption from the Intestine," Academic Press, London, 1964, p. 218.