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Studies on Synthetic Sweetening Agents. XVII.[1] Metabolism of Sodium Cyclamate. (6). Influences of Neomycin and Sulfaguanidine on Metabolism of Sodium Cyclamate

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For the purpose of elucidating the metabolic sites of sodium cyclamate (CHS-Na), the influences of neomycin and sulfaguanidine on the metabolism of CHS-Na were investigated in rabbits, rats, and mice.

Neomycin and sulfaguanidine caused a conspicuous decrease in the urinary excretion of CHS-Na metabolites in rats and mice. However the excretion of CHS-Na metabolites in rabbits did not decrease by administration of neomycin or sulfaguanidine. Also, CHS-Na metabolites were found in the urine of rabbits and rats following intravenous dose of CHS-Na. These results demonstrate that the metabolism of CHS-Na in the rat and mouse is predominantly carried out in the intestines, probably by the microbial flora, and that CHS-Na metabolism in the rabbit takes place mainly in the liver.

It has been recently shown by many investigations that cyclamate can be metabolized to cyclohexylamine by humans and animals. Some workers[3-6] have reported on the metabolism of the cyclamate in rats and guinea pigs, and they suggested that the conversion of cyclamate to cyclohexylamine was probably carried out by the microbial flora in the intestines of the animals. However, the authors[1,7] reported that cyclohexylamine, cyclohexanone, and cyclohexanol were found in the *in vitro* metabolism of sodium cyclamate (CHS-Na) by liver homogenates of rabbit and rat. Accordingly, studies were conducted in rabbits, rats, and mice to clarify the metabolic sites of CHS-Na.

In the present paper, the *in vivo* metabolism of CHS-Na was investigated in rabbits, rats, and mice, which had been given orally neomycin sulfate or sulfaguanidine to reduce the microbial flora in the intestinal tracts of those animals. In addition, CHS-Na metabolism was investigated in rabbits and rats by an intravenous injection of CHS-Na.

Experimental

Materials——Pure sample of CHS-Na was obtained by repeated recrystallization of reagent grade one, and dried *in vacuo* for 6 hr. Cyclohexylamine, cyclohexanol, cyclohexanone, isoamylacetate, and *n*-butylether were purified by distillation of commercial products of reagent grade. Neomycin sulfate and sulfaguanidine were of J.P. VII grade. Other chemicals used were of reagent grade.

Test Animals, Administration of Drugs, and Collections of Urine——Male rabbits weighing about 3 kg, male Wistar rats weighing 150—250 g, and male mice of dd-strain, weighing about 20 g, were kept on the solid food and water in individual cages. But five mice were maintained as one group. The drugs dissolved or suspended in 50 ml (for rabbit), 1—4 ml (for rat), and 0.2 ml (for mouse) of water were given by a stomach tube. Urine was collected in a flask containing toluene for preventing putrefaction.

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Apparatus and Conditions of Gas Liquid Chromatography——The apparatus and conditions of gas liquid chromatography were same as in the previous paper.[8]

Determination of Unchanged CHS-Na in Urine——Unchanged CHS-Na, which was excreted in the urine of rabbits, rats, and mice receiving CHS-Na, was determined according to the gas chromatographic method reported in the previous paper.[8]

Determinations of Cyclohexylamine, Cyclohexanone, Cyclohexanol, and Cyclohexylglucuronide in Urine Cyclohexylamine, cyclohexanone, cyclohexanol and its glucuronide which were excreted in the urine of rabbits, rats, and mice receiving CHS-Na, were determined by the gas chromatographic methods described in the previous paper of this series.[9]

Biliary Excretion of CHS-Na in Rabbits and Determination of Unchanged CHS-Na in the Bile——Male rabbits weighing about 3 kg were anesthetized with urethan (1.0 g/kg *i.p.*), supplemental doses being administered as needed. The bile duct was surgically exposed by a midline abdominal incision and cannulated with polyethylene tubing (PE-10). A dose of 500 mg of CHS-Na dissolved in 5 ml of water was administered intravenously, and bile samples were collected at 1 hr intervals for 4 hr after administration of the durg. Unchanged CHS-Na in the bile samples was determined according to the gas chromatographic method reported in the previous paper.[8]

Result and Discussion

CHS-Na (rabbit, 200 mg/kg; rat, 100 mg/animal; mouse, 10 mg/animal) was administered orally together with neomycin sulfate (rabbit, 100 mg/kg; rat, 30 mg/animal; mouse, 2 mg/animal) to rabbits, rats, and mice which had been pretreated with the same dose of

Table I. Metabolism of CHS-Na in Rabbits Pretreated with Neomycin or Sulfaguanidine

Condition	Exp. No.	% of metabolites excreted[a]					Recovery (%) of unchanged CHS-Na
		Cyclohexyl-amine	Cyclohexa-none	Cyclohexa-nol	Conjugated cyclohexanol	Total	
Control	1	0.0079	0.0002	0.0012	0.0116	0.0209	88.1
	2	0.0116	0.0002	*b*)	0.0069	0.0187	84.0
	3	0.0040	0.0016	0.0001	0.0123	0.0180	78.4
	4	0.0036	*b*)	*b*)	0.0104	0.0140	93.6
	mean±	0.0068±	0.0005±	0.0003±	0.0103±	0.0179±	87.5±
	S.D.	0.0033	0.0006	0.0005	0.0021	0.0025	5.8
Pretreated with neomycin	1	0.0098	0.0020	0.0002	0.0265	0.0385	85.7
	2	0.0050	0.0003	0.0002	0.0095	0.0150	80.0
	3	0.0034	0.0016	*b*)	0.0197	0.0247	91.1
	4	0.0048	0.0005	*b*)	0.0153	0.0206	96.5
	mean±	0.0058±	0.0011±	0.0001±	0.0178±	0.0247±	90.9±
	S.D.	0.0024	0.0007	0.0001	0.0062	0.0087	6.7
Control	1	0.0090	0.0009	0.0017	0.0148	0.0264	103.0
	2	0.0093	0.0002	*b*)	0.0152	0.0247	79.7
	3	0.0017	0.0001	0.0001	0.0125	0.0144	62.0
	4	0.0035	0	0	0.0103	0.0138	84.0
	mean±	0.0059±	0.0003±	0.0005±	0.0132±	0.0198±	82.2±
	S.D.	0.0033	0.0003	0.0007	0.0019	0.0057	14.6
Pretreated with sulfa-guanidine	1	0.0108	0.0005	0.0003	0.0142	0.0258	95.5
	2	0.0017	0.0006	0.0007	0.0062	0.0092	49.5
	3	0.0008	0.0005	0.0002	0.0097	0.0112	—
	4	0.0025	0.0077	0.0030	0.0101	0.0233	92.5
	mean±	0.0040±	0.0023±	0.0011±	0.0101±	0.0174±	79.2±
	S.D.	0.0040	0.0031	0.0011	0.0028	0.0072	21.0

a) The percentage of each metabolite is given in terms of CHS-Na equivalent.
b) indicate extremely small percentage
dose: CHS-Na (200 mg/kg), neomycin sulfate (100 mg/kg), sulfaguanidine (100 mg/kg)

8) H. Ichibagase, S. Kojima, K. Inoue, and A. Suenaga, *Chem. Pharm. Bull.* (Tokyo), **20**, 175 (1972).
9) S. Kojima and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **17**, 2620 (1969).

neomycin sulfate as described above for 6 days. Also, sulfaguanidine (rabbit, 100 mg/kg; rat, 30 mg/animal) was given orally to rabbits and rats for 5 days, and on the 5th day of sulfaguanidine administration CHS-Na (rabbit, 200 mg/kg; rat, 100 mg/animal) was given at the same time. Then CHS-Na metabolites were determined in 24 hours-urine of rabbits, rats, and mice following the administration of CHS-Na with and without neomycin sulfate or sulfaguanidine. As shown in Table I, the amounts of CHS-Na metabolites in the urine of rabbits pretreated with neomycin sulfate or sulfaguanidine were almost the same amount as those in control rabbits. On the other hand, as shown in Tables II and III, the urinary excretion of cyclohexylamine as a metabolite of CHS-Na in rats and mice pretreated with

Table II. Metabolism of CHS-Na in Rats Pretreated with Neomycin or Sulfaguanidine

Condition	Exp. No.	% of metabolites excreted[a]					Recovery (%) of unchanged CHS-Na
		Cyclohexyl-amine	Cyclohexa-none	Cyclohexa-nol	Conjugated cyclohexanol	Total	
Control	1	0.0085	0.0009	0.0027	0.0079	0.0200	8.7
	2	0.0103	0.0005	0.0021	0.0049	0.0178	6.8
	3	0.0103	0.0008	0.0020	0.0093	0.0225	8.9
	mean±	0.0097±	0.0007±	0.0023±	0.0074±	0.0201±	8.1±
	S.D.	0.0008	0.0002	0.0003	0.0018	0.0019	1.0
Pretreated with neomycin	1	0	0	0	0.0039	0.0039	14.1
	2	0	0.0017	0	0.0046	0.0063	15.2
	3	0.0014	0	0	0.0060	0.0074	10.5
	mean±	0.0005±	0.0006±	0	0.0048±	0.0059±	13.5±
	S.D.	0.0006	0.0008		0.0008	0.0014	2.0
Control	1	0.0119	0.0030	0.0008	0.0092	0.0248	9.8
	2	0.0106	0	0	0.0360	0.0466	9.1
	3	0.0062	0.0005	0.0011	0.0055	0.0134	11.5
	mean±	0.0096±	0.0012±	0.0006±	0.0169±	0.0283±	10.1±
	S.D.	0.0024	0.0013	0.0004	0.0135	0.0137	1.0
Pretreated with sulfa-guanidine	1	0.0112	0.0014	*b*)	0.0049	0.0175	9.5
	2	0.0032	0	0.0011	0.0082	0.0125	16.5
	3	0.0086	0.0006	*b*)	0.0054	0.0146	6.1
	mean±	0.0077±	0.0007±	0.0004±	0.0061±	0.0149±	10.6±
	S.D.	0.0033	0.0005	0.0005	0.0014	0.0020	4.2

a) The percentage of each metabolite is given in terms of CHS-Na equivalent.
b) indicate extremely small percentage
dose: CHS-Na (100 mg/animal), neomycin sulfate (30 mg/animal), sulfaguanidine (30 mg/animal)

Table III. Metabolism of CHS-Na in Mice Pretreated with Neomycin

Condition	Exp. No.	% of metabolites excreted[a]					Recovery (%) of unchanged CHS-Na
		Cyclohexyl-amine	Cyclohexa-none	Cyclohexa-nol	Conjugated cyclohexanol	Total	
Control	1	0.0176	0	0	0	0.0176	16.0
	2	0.0212	0.0044	0	0	0.0256	14.1
	3	0.0214	0.0030	0	0	0.0244	16.4
	mean±	0.0201±	0.0025±	0	0	0.0226±	15.5±
	S.D.	0.0017	0.0018			0.0035	1.0
Pretreated with neomycin	1	0	0	0	0.0042	0.0042	19.9
	2	0	0.0034	0	0	0.0034	17.1
	3	0	0.0050	0	0	0.0050	18.0
	mean±	0	0.0028±	0	0.0014±	0.0042±	18.3±
	S.D.		0.0021		0.0020	0.0007	1.2

a) The percentage of each metabolite is given in terms of CHS-Na equivalent. Five mice were used as one group.
dose: CHS-Na (50 mg/one group), neomycin sulfate (10 mg/one group)

neomycin sulfate decreased to undetectable levels, and also the total amounts of CHS-Na metabolites such as cyclohexylamine, cyclohexanone, cyclohexanol and its glucuronide excreted in the urine were about quarter those in control animals. Sulfaguanidine also caused a decrease in the excretion of CHS-Na metabolites in the rats (see Table II).

As can be seen from Tables I, II and III, and from Fig. 1, recovery percentages of unchanged CHS-Na in the urine of rabbits, rats, and mice pretreated with neomycin or sulfaguanidine were almost the same with those in the animals received CHS-Na alone. Accordingly, these results indicate that a decrease in the urinary excretion of CHS-Na metabolites in rats and mice pretreated with neomycin or sulfaguanidine is not attributed to the inhibitory effect of neomycin or sulfaguanidine on the gastrointestinal absorption of CHS-Na in the animals.

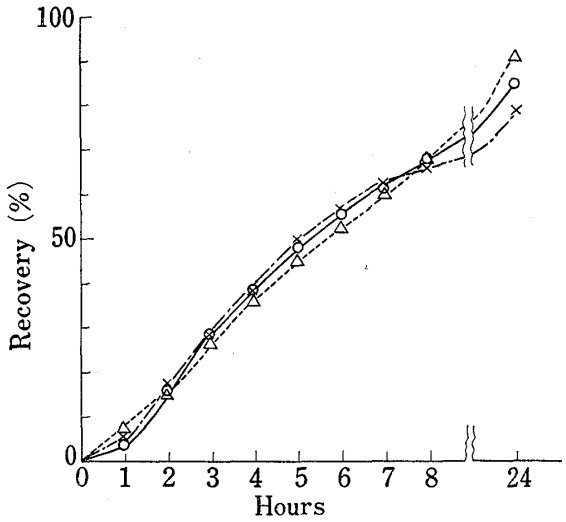

Fig. 1. Urinary Recovery Curves for CHS-Na after Oral Administration of CHS-Na (200 mg/kg) in Rabbits Pretreated with Neomycin (100 mg/kg) or Sulfaguanidine (100 mg/kg)

Recovery (%) is expressed as the mean value of 4 experiments.
——○——: CHS-Na alone
------△------: pretreated with neomycin sulfate
---×---: pretreated with sulfaguanidine

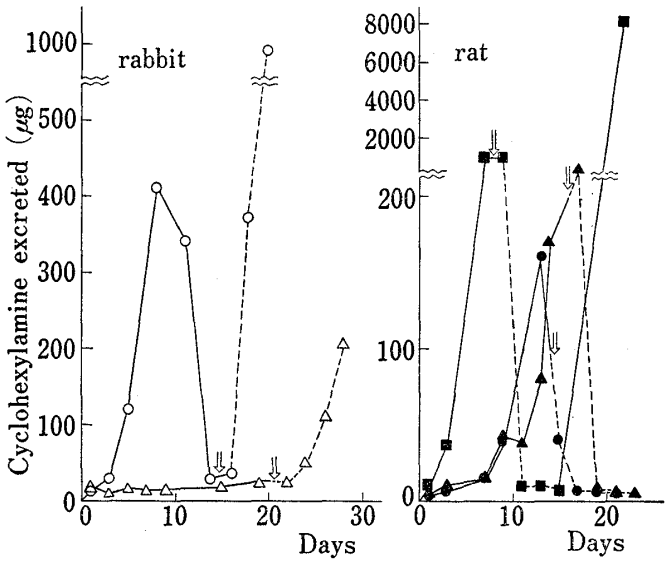

Fig. 2. Effect of Neomycin on the Metabolism of CHS-Na in Rabbits and Rats following Prolonged Administration of CHS-Na

The beginning of administration of neomycin together with CHS-Na is showed by an arrow.
The solid lines indicate the urinary excretion of cyclohexylamine following prolonged administration of CHS-Na alone and the dotted lines the urinary amount of cyclohexylamine after administration of neomycin together with CHS-Na.
dose: CHS-Na (rabbit, 1g/animal; rat, 100 mg/animal), neomycin (rabbit, 400 mg/animal; rat, 30 mg/animal)

Moreover, the influence of neomycin on the CHS-Na metabolism was investigated by oral administration of CHS-Na (rabbit, 1 g/animal; rat, 100 mg/animal) together with neomycin (rabbit, 400 mg/animal; rat, 30 mg/animal) for 7 days to rabbits and rats, which had continued to receive CHS-Na (rabbit, 1 g/animal/day; rat, 100 mg/animal/day) orally and had excreted a considerable amount of cyclohexylamine as a metabolite of CHS-Na. The results of quantitative determination of urinary cyclohexylamine as a metabolite of CHS-Na were shown in Fig. 2. The amount of cyclohexylamine in the urine of rats conspicuously decreased after administration of neomycin, resulting in undetectable levels. And the urinary excretion of cyclohexylamine increased after stopping administration of neomycin. On the other hand, the urinary excretion of cyclohexylamine in rabbits did not decrease by administration of neomycin.

From the above results, it is presumed that the conversion of CHS-Na into cyclohexylamine in rats and mice is predominantly carried out by the gut flora and that cyclohexylamine produced is almost completely absorbed from the intestines and then excreted in the urine. On the contrary the metabolism of CHS-Na in rabbits may be little carried out in the intestines.

Furthermore, in order to elucidate whether CHS-Na could be also metabolized by parenteral administration of the drug, CHS-Na (rabbit, 500 mg/animal; rat, 50 mg/animal) was administered intravenously to rabbits and rats, and CHS-Na metabolites in 24 hours-urine were determined by gas chromatographic method. As shown in Table IV, CHS-Na metabolites such as cyclohexylamine, cyclohexanone, and cyclohexanol were distinctly found in the urine of rabbits and rats, although urinary excretion of those metabolites was small amount. In addition, in an *in situ* biliary excretion experiment in rabbits, an average of only 0.55% of the intravenous dose (500 mg/animal) was excreted into the bile during the first four hours (see Table V). Thus, the bile is not an important route of excretion of CHS-Na in the rabbit as Wallace, *et al.*[5] have already investigated in rats. The large amounts of CHS-Na metabolites excreted in the urine by the rabbits and rats following intravenous administration of CHS-Na are probably the result of CHS-Na metabolism in any tissues other than the intestines and not by degradation in the intestines. In the previous papers,[1,7] also, the authors reported that cyclohexylamine, cyclohexanone, and cyclohexanol were found as the metabolic products of CHS-Na in the *in vitro* experiment using rabbit and rat liver, and that the produced amounts of CHS-Na metabolites in the rat liver were very small compared with those in the rabbit liver.

Table IV. Metabolism of CHS-Na in Rabbits and Rats following Intravenous Administration of CHS-Na

	Exp. No.	% of metabolites excreted			
		Cyclohexylamine	Cyclohexanone	Cyclohexanol	Total metabolites
Rabbit	1	0.0184	0.0012	0.0004	0.0200
	2	0.0120	0.0019	0.0040	0.0179
Rat	1	0.0288	0.0028	0.0024	0.0340
	2	0.0029	0.0024	0.0028	0.0081
	3	0.0029	0.0024	0.0026	0.0079

The percentage of each metabolite is given in terms of CHS-Na equivalent.
dose of CHS-Na: rabbit (500 mg/animal), rat (50 mg/animal)

Table V. Cumulative Excretion of Unchanged CHS-Na by Rabbits with Cannulated Bile Ducts after Intravenous Dose of CHS-Na (500 mg/animal)

Exp. No.	% of unchanged CHS-Na excreted, hr			
	1	2	3	4
1	0.242	0.275	0.282	0.287
2	0.468	0.650	0.752	0.805
3	0.344	0.375	0.380	0.384
4	o.198	0.230	0.255	0.273
5	0.658	0.881	0.975	1.018
mean	0.382	0.482	0.529	0.553

Accordingly, the above results establish that the metabolism of CHS-Na in the rat and mouse is predominantly carried out in the intestines, probably by the microbial flora present there, and that CHS-Na metabolism in the rabbit takes place mainly in the liver.