[Chem. Pharm. Bull. 14(9) 959~965 (1966)] UDC 615.756.8-07 : 614.31

130. Shoji Kojima, Hisashi Ichibagase,*1 and Sadao Iguchi*2 : Studies on Synthetic Sweetening Agents. Ⅵ.*3 Absorption and Excretion of Sodium Cyclamate. (1).

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Sodium cyclamate (CHS-Na) has recently been made available as a noncaloric sweetening agent. The absorption and the excretion of CHS-Na were studied by Audrieth, *et al.*[1], Taylor, *et al.*[2], and Schoenberger, *et al.*[3] These studies were carried out to estimate the urinary excretion of CHS-Na and the distribution of the drug in tissue after oral administration or injection in humans, rats, rabbits, and dogs.

A new colorimetric method for the determination of CHS-Na was reported previously by the authors.[4] In this paper, the new colorimetric method was used for estimating the amount of urinary unchanged CHS-Na, and the absorption of CHS-Na was calculated from the excretion data. Furthermore, the effects of various kinds of compounds contained in natural foods on the absorption and the excretion of CHS-Na were investigated.

Experimental

Animals——Male rabbits weighing 2.5 to 3.4 kg. were fasted for about 20 hours prior to the experiments.

Methods of Administration——Orally : 600 mg. of CHS-Na was administered to rabbits with or without other compounds such as caffein, theophylline, theobromine, albumin, casein, and citric acid. All the compounds tested were administered orally by using Nelaton's catheter in the forms of solution or suspension in 20 ml. of water. In human, 100 ml. of water solution which contained 1.0 or 5.0 g. of CHS-Na was administered orally.

Intravenously injection : 100 mg. of CHS-Na per kg. body weight was injected in the form of 10 w/v% water solution through ear-vein of rabbit.

Collections of Urine Samples——In rabbits, the urine samples were collected by using Nelaton's catheter at the desired time until 8 hr. after administration of the drug, thereafter the urine was collected in the flask which contained toluene for preventing putrefaction. In humans, the urine samples were collected at the desired time after administration of the drug.

Analytical Methods——The colorimetric method with quinhydrone : Urine containing CHS-Na was diluted with water to a concentration of 50 to 400 μg. per ml. of CHS-Na. Each urine sample was analyzed for CHS-Na according to the colorimetric method B which was reported previously.[4] The recovery of CHS-Na which was added to the urine was 99±3%.

Chelatometric titration method : A known amounts of CHS-Na was added to the urine from human and rabbit, and the recovery of CHS-Na was estimated according to the chelatometric titration method which was described in J.P. Ⅶ. In the range between 5 to 20 mg. of CHS-Na, the method showed satisfactory results with precision (100±3%), but the precision was diminished as the amount of CHS-Na was decreased.

Precipitate Formation with Albumin or Casein——The sample solutions were prepared by dissolving the various amount of CHS-Na in 0.1% water solution of albumin or casein. The pH of the sample solutions was adjusted to the desired pH by adding 0.1*N* or 1*N* HCl, and was estimated with Horiba pH meter

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*3 Part V. H. Ichibagase, S. Kojima, M. Ichikawa : Yakugaku Zasshi, **84**, 707 (1964).

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2) J. D. Taylor, R. K. Richards, J. C. Davin : Proc. Soc. Exp. Biol. Med., **78**, 530 (1951).
3) J. A. Schoenberger, D. M. Rix, A. Sakamoto, J. D. Taylor, R. M. Kark : Am. J. Med. Sci., **225**, 551 (1953).
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Model-H. Immediately after shaking vigorously, the absorbancy of the mixture was measured at the wave length of 650 mμ with Shimadzu spectrophotometer Model QR-50 using a 10 mm. cell.

Results and Discussion

Comparison of the Colorimetric Method with the Official Method (Chelatometric Titration Method) of the Determination of CHS-Na in Urine

In order to test the precision of the both methods for estimating urinary CHS-Na, the drug was administered orally to rabbits. Then the urine sample were collected at the desired period of time. The values obtained by both methods were different (Table I). The differences were supposed to be due to the blank values of the chelatometric method. Thus the blank values were estimated as follow; a rabbit was administered orally with 20 ml. of water, then the urine samples were collected at various times. Each blank urine was diluted to 10 times with water, and the diluted urine was used for both the official chelatometric titration and the colorimetric method with quinhydrone. Results obtained by these experiments show that the blank values by the official method are variable as shown in Table II. Thus the colorimetric method with quinhydrone was applied the purpose of estimating urinary CHS-Na.

Table I. Recovery of CHS-Na and ml. Urine excreted to Various Times after Oral Administration of CHS-Na to Rabbits (600 mg. CHS-Na per Animal)

Time (hr.)	Urine (ml.)	Recovery (%)	
		Chelatometric titration method	Colorimetric method with quinhydrone
1	8.8	1.6	1.1
2	4.0	10.0	7.7
3	27.5	40.6	26.8
4	16.5	57.8	38.8
5	7.5	70.6	46.0
6	3.8	83.3	56.5
24	185.0	164.0	88.9

Table II. Blank Values in Rabbit Urine

Time (hr.)	Chelatometric titration method (ml. 0.01*M* EDTA)[a]	Colorimetric method with quinhydrone (Absorbancy at 495 mμ)[b]
0	25.80	0.025
1	23.69	0.022
2	21.39	0.019
3	20.86	0.014
4	20.74	0.014
5	20.26	0.016
6	20.30	0.014
8	19.70	0.026
24	21.75	0.029

a) Twenty ml. of diluted blank urine was used to chelatomeric titration method.
b) Two ml. of diluted blank urine was used to colorimetric method with quinhydrone.

Absorption and Excretion of CHS-Na in Rabbits

The excretion of intravenously injected CHS-Na to rabbits is shown in Table III. The results show that 81.5 to 98.0% of the drug was eliminated within 24 hours after

injection and are in good agreement with those which were reported by Schoenberger, *et al.*[3] in human subject.

Absorption of CHS-Na was studied after administering orally to rabbits. The mean cumulative data of excretion are shown in Table Ⅳ and are plotted against to the time as shown in Fig. 1.

Table Ⅲ. Urinary Excretion of CHS-Na per 24 Hours in Rabbits following a Single Intravenous Dose of 100 mg. per kg. wt.

Rabbit	1	2	3	4	5	6		
Body weight (kg.)	2.6	3.0	2.5	3.4	2.5	2.5		
Recovery (%)	95.2	81.5	89.6	98.0	91.2	95.3	mean	91.8

Table Ⅳ. Cumulative Excretion of CHS-Na at Various Times after Oral Administration of 600 mg. Dose

Rabbit	Body weight (kg.)	mg. excreted, hr.							
		1	2	3	4	5	6	8	24
1	2.9	18.5	65.9	136.1	205.3	275.7	335.9	414.9	517.9
2	3.0	11.3	49.3	132.9	212.9	287.6	347.0	447.9	550.2
3	3.3	2.7	41.0	97.8	168.0	234.0	291.4	375.9	495.9
4	3.0	25.9	110.7	179.3	247.6	294.6	336.9	391.1	470.8
mean		14.6	66.7	136.5	208.4	273.0	327.8	407.4	508.7
mean recovery (%)		2.4	11.1	22.7	34.7	45.5	54.6	67.9	84.8

The rate of excretion was obtained from the mean cumulative excretion curve on Fig. 1. The rates obtained by this graphical determination are shown in the second column of Table Ⅴ.

When the logarithm of excretion rate is plotted as a function of time, the graph shows a straight line after 3 hours as shown in Fig. 2 and the results indicate that the excretion of CHS-Na is the first order.

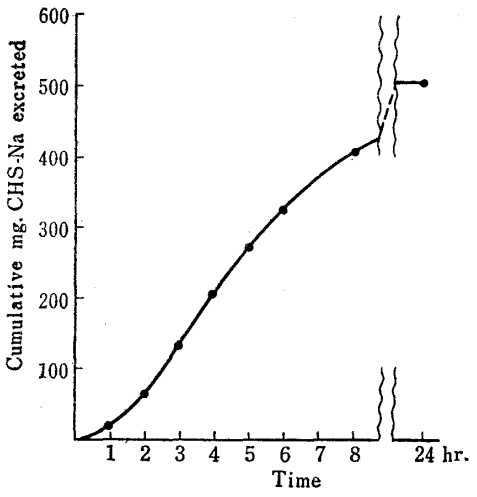

Fig. 1. Mean Cumulative Excretion Curve for CHS-Na after Oral Administration of 600 mg. Doses in Rabbits

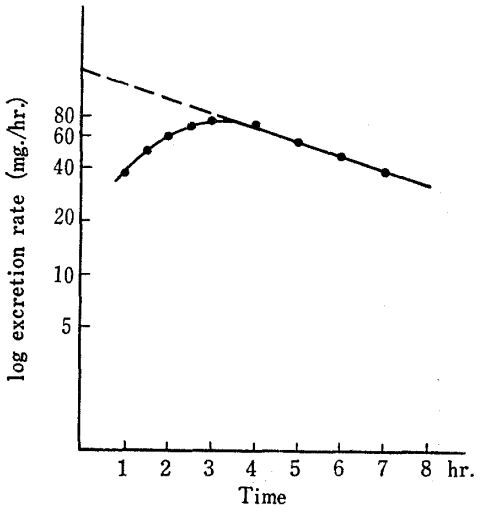

Fig. 2. Logarithm of Excretion Rate *vs.* Time after Oral Administration of 600 mg. CHS-Na

The excretion rate constant (k) can be determined from the slope of the straight line. The half-life ($t_{1/2}$) for elimination of CHS-Na can be calculated from the equation (1).

$$t_{1/2}=\frac{0.693}{k} \qquad (1)$$

The excretion rate constant thus obtained was 0.183 hours^{-1}. The value of half-life obtained by use of this constant was 3.9 hours.

Table V. Excretion Rate and Absorption Rate from Urinary Excretion Data of CHS-Na

Time (hr.)	Excretion rate (mg./hr.)	Derivatives of excretion rate (mg./hr.^{2})	Absorption rate from excretion data (mg./hr.)
0.5	15	41	260
1.0	38	38	267
1.5	52	23	192
2.0	62	18	174
2.5	70	13	153
3.0	75	0	81
4.0	71		
5.0	58		
6.0	49		
7.0	39		

The relationship between the rate of absorption and the excretion is expressed in the equation (2) by Nelson, *et al.*[5]

$$\frac{dA}{dt}=\frac{1}{f}\left(\frac{dU}{dt}+\frac{1}{k}\cdot\frac{d^2U}{dt^2}\right) \qquad (2)$$

where $\frac{dA}{dt}$ is the absorption rate at time t in mg./hr., f is the fraction of the drug absorbed which is excreted unchanged in the urine, $\frac{dU}{dt}$ is the excretion rate, $\frac{d^2U}{dt^2}$ is the second derivative obtained graphically from a plot of excretion rate *vs.* time (hr.), and k is the excretion rate constant. The values of the second derivative obtained are shown in the third column of Table V. The excretion rate has been listed in the second column of Table V, and the excretion rate constant has been shown previously. The value of f was calculated as 0.92 from the data in Tables III and IV. The calculated absorption rate in mg./hr. at various times is listed in column 4 of Table V.

Effect of Other Compounds on Absorption and Excretion of CHS-Na in Rabbits

The effects of caffeine, theophylline, theobromine, albumin, casein, and citric acid on the absorption and excretion of CHS-Na were studied, and CHS-Na was administered orally with those compounds in rabbits. The excretion data of CHS-Na are shown in Table VI. The cumulative exeretion curve are shown on Fig. 3. The cumulative excretion amounts of CHS-Na, if it was administered with caffeine or citric acid, were greater than those excreted after single administration of CHS-Na. In these experiments, the relation between the logarithm of excretion rate and the period of time shows a straight line as shown in Fig. 4, and the excretion rate constant obtained from the

5) E. Nelson, I. Schaldemose: J. Am. Pharm. Assoc., 48, 489 (1959).

slope of the straight line and the half-life for elimination calculated by the equation (1) were almost the same as in the case of administration of CHS-Na without any other compounds as shown in Table Ⅶ. Consequently, caffeine and citric acid had no significant effect on the excretion of CHS-Na.

Table Ⅵ. Cumulative mg. CHS-Na excreted to Various Times after Oral Administration of 600 mg. CHS-Na with Other Compounds in Rabbits

	Dose (mg.)	Numbers of exp.	Body wt. (kg.)	mg. excreted, hr. 1	2	3	4	5	6	8	24
Caffeine	150	4	2.9~3.4	21.9	91.9	168.2	248.2	313.1	372.1	466.0	573.5
Theophylline	150	4	2.8~3.4	16.3	59.9	119.7	190.8	254.8	304.6	385.3	501.2
Theobromine	150	3	2.6~3.4	8.8	55.5	125.7	202.3	266.5	317.7	403.2	544.4
Albumin	400	4	2.6~3.3	12.2	71.4	153.7	226.0	292.1	340.0	411.6	528.4
Casein	400	5	2.6~3.3	13.3	65.3	139.0	208.4	276.1	332.0	413.6	546.7
Citric acid	2000	3	2.8~3.0	12.5	79.5	157.8	242.7	316.4	381.1	472.2	573.3

Table Ⅶ. Excretion Rate Constant and Half-life for CHS-Na in Rabbits following Oral Administration of CHS-Na with Other Compounds

Other compd.	k (hr.$^{-1}$)	$t_{1/2}$ (hr.)	Other compd.	k (hr.$^{-1}$)	$t_{1/2}$ (hr.)
Without	0.183	3.9	Albumin	0.193	3.6
Caffeine	0.173	4.0	Casein	0.189	3.7
Theophylline	0.177	3.9	Citric acid	0.190	3.7
Theobromine	0.184	3.8			

The absorption rates of CHS-Na in the presence of caffeine and citric acid are shown in Table Ⅷ. From the results obtained, it is clear that the increase of the absorption rate of CHS-Na is observed during the first 1.5 hours following administration with caffeine or citric acid. This fact suggests that caffeine and citric acid increase the absorption of CHS-Na.

Furthermore, Ichibagase, *et al.*[6] reported that albumin and casein were precipitated by CHS-Na from the water solutions. The precipitate formation by albumin or casein with various concentrations of CHS-Na was tested by changing the pH of the medium. As shown in Fig. 5, the results indicated that the precipitate formation was affected by changing pH, and was the greatest at pH 3.0 in albumin, and at pH 4.0 in casein, respectively. The absorption of CHS-Na, however, was not affected by those compounds as shown in Table Ⅵ.

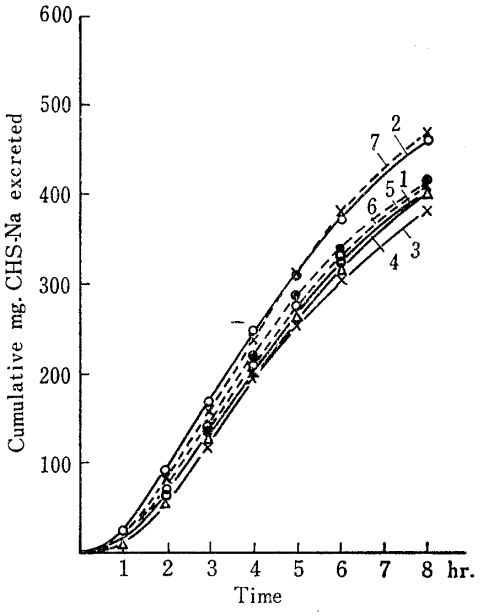

Fig. 3. Mean Cumulative Excretion Curves for CHS-Na following Oral Administration of 600 mg. Doses with Other Compounds

Curve 1: CHS-Na
2: 〃 + Caffeine
3: 〃 + Theophylline
4: 〃 + Theobromine
5: 〃 + Albumin
6: 〃 + Casein
7: 〃 + Citric acid

6) H. Ichibagase, *et al.*: Unpublished.

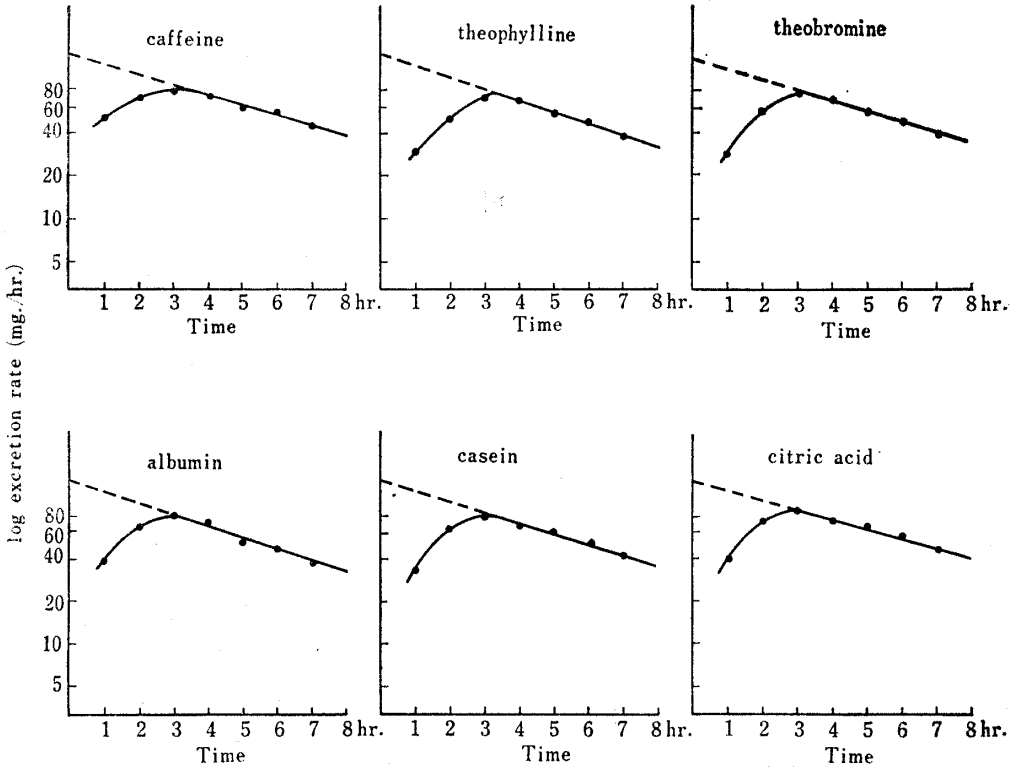

Fig. 4. Logarithm of Excretion Rate *vs.* Time after Oral Administration of 600 mg. CHS-Na with Other Compounds

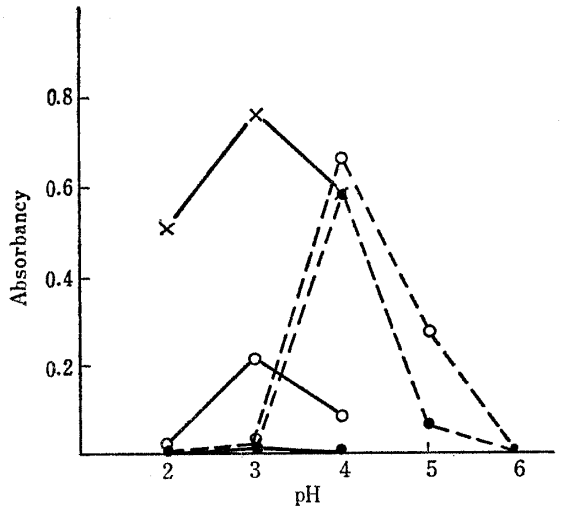

Fig. 5. Precipitate Formation by Albumin or Casein with CHS-Na

●—● 0.1% Albumin +1% CHS-Na
○—○ 〃 +2% 〃
×—× 〃 +3% 〃
●·····● 0.1% Casein +0.3% CHS-Na
○·····○ 〃 +0.5% 〃

Table Ⅷ. Absorption Rate of CHS-Na from Urinary Excretion Data following Oral Administration of CHS-Na with Caffeine or Citric Acid

	Time (hr.)	Excretion rate (mg./hr.)	Derivatives of excretion rate (mg./hr.²)	Absorption rate from excretion data (mg./hr.)
with caffeine	0.5	22	72	476
	1.0	55	41	317
	1.5	69	15	170
	2.0	74	7	124
	2.5	76	5	114
	3.0	79	0	86
with citric acid	0.5	13	41	249
	1.0	40	81	506
	1.5	67	33	262
	2.0	77	13	156
	2.5	82	11	152
	3.0	85	0	92

Absorption and Excretion of CHS-Na in Human

The absorption of CHS-Na in humans was studied for three subjects following oral administration. The results are shown in Table Ⅸ. The mean values of urinary excretion of CHS-Na obtained by the administration of a single oral dose of 5.0 g. and 1.0 g. were 2.42 g. (48%) and 0.40 g. (40%) per 24 hours respectively. The results of present studies are in good agreement with those reported by Schoenberger, *et al.*[3].

Table K. Cumulative mg. CHS-Na excreted to Various Times following Oral Administration of 5.0 g. or 1.0 g. Dose in Humans

Dose (g.)	Subject[a]	mg. excreted, hr. 1	2	3	4	5	6	24
5.0	S.K.(33～50) ♂	132	267	365	454	580	775	3100
〃	S.H.(23～52) 〃	—	—	—	—	—	—	2390
〃	M.D.(22～60) 〃	—	—	—	—	—	—	1765
1.0	S.K.(33～50) 〃	22	50	73	91	—	154	382
〃	S.H.(23～52) 〃	—	—	—	—	—	—	420

a) Bracketed quantities are subject's age in years followed by his weight in kilograms.

Summary

Studies on the absorption and excretion of CHS-Na in rabbit and human are reported in this paper.

The urinary excretion rate of CHS-Na was determined after oral administration to rabbit, and the absorption rate was calculated from the excretion data.

And the absorption of CHS-Na was studied after oral administration with the compounds such as caffeine, theophylline, theobromine, albumin, casein, and citric acid, and it was suggested that the absorption of CHS-Na was accelerated in the presence of caffeine and citric acid.

(Received January 6, 1966)

[Chem. Pharm. Bull. 14(9) 965～971 (1966)]

UDC 615 : 756.8-07 : 614.31

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In the previous report,[*3] the absorption and the excretion of sodium cyclamate (CHS-Na) were studied in rabbit and human.

The present report describes the absorption of CHS-Na from the stomach or the small intestine of rat *in situ*. And the absorption experiments of saccharin and dulcin were also carried out with the same method and were compared with that of CHS-Na.

In those experiments described above, the absorption of CHS-Na in rat *in situ* was shown to be less than *in vivo*. In order to investigate the causes of the reduction of the absorption, the effects of pentobarbital, which was used as anesthetic in those experiments, on the absorption of CHS-Na were also studied in rabbit and rat.

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