PHARMACOLOGICAL STUDIES OF GARDENIA FRUIT. V.¹⁾ MECHANISMS OF INHIBITORY EFFECT OF GENIPIN ON GASTRIC ACID SECRETION AND ITS FACILITATORY EFFECT ON BILE SECRETION IN RATS

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In the experiment on continuous perfusion of rat stomach *in vivo*, genipin inhibited only the gastric acid secretion induced by carbachol, but not by tetragastrin, or histamine. In the experiment on isolated organs, genipin showed a weak competitive *anti*-acetylcholine action on the intestinal contraction. Based on these facts and results reported previously, it is conceivable that *anti*-cholinergic action at least partly contributes to the genipin-induced inhibitory effect on gastric functions.

Erythritol clearance was increased with the increase in bile flow by administration of genipin. Genipin showed a significant choleretic action, and just then the concentration of biliary bile acid was decreased inversely. In the relationship between bile flow and biliary bile acid excretion rate, the slope of regression line obtained from genipin-treated group was not significantly different from that of control, and these lines were approximately parallel to each other. Genipin did not affect the concentration of sodium, potassium, chloride, or bicarbonate in the bile collected during the initial stage, in which bile flow was increased, after administration. It is concluded from these results that genipin-induced choleretic action proceeds by a mechanism wherein water is driven along osmotic gradient which originates in the transport of bile acid-independent fraction from hepatocytes into canaliculi, mainly through active Na⁺ transport.

Keywords—Gardenia fruit; choleretic action; genipin; erythritol clearance; inhibitory effect on gastric acid secretion; bile acid-independent fraction; active Na⁺ transport; anti-ACh action

We have reported earlier that geniposide, the iridoid glucoside component contained in gardenia fruit, showed a delayed and prolonged choleretic action, while genipin, its aglucone, showed a rapid choleretic action of about the same degree as sodium dehydrocholate, an inhibitory action on gastric juice secretion, and on spontaneous as well as pilocarpine-induced gastric contraction.

We have concluded that, when geniposide was ingested, it was hydrolyzed in the intestine and genipin formed was absorbed in portal blood together with unchanged geniposide and exerted its choleretic action in the liver.

The present study was undertaken to examine mechanisms of the inhibitory effect of genipin on gastric acid secretion and its facilitatory effect on bile secretion in rats.

MATERIALS AND METHODS

Animals — Male ddY strain mice, male Wistar strain rats, and male Hartley strain guinea pigs were used.

Materials — Genipin was dissolved in physiological saline just before the administration.

Chemicals — Sodium dehydrocholate was purchased from Dainippon Pharmaceutical Co., tetragastrin from San-a Chemical Ind. Co., car-

bamylcholine chloride (carbachol) from Tokyo Kasei Ind., histamine dihydrochloride (Hist) and 5-hydroxytryptamine sulfate (5-HT) from Wako Pure Chemical Ind., acetylcholine chloride (ACh) from Daiichi Pharmaceutical Co., pilocarpine hydrochloride (Pilo) from Sanko Pharmaceutical Co., and erythritol [$U\text{-}^{14}\text{C}$] from Radiochemical Centre, Amersham, England.

Methods — (1) Effect on Gastric Acid Secretion in Rats: Rat weighing 200–230 g were used. Continuous perfusion of rat stomach *in vivo* was performed according to the method of Ghosh and Schild.²⁾ Under anesthesia with urethan (1.5 g/kg, *s.c.*) after being fasted for 18 hr, rats were laparotomized, polyethylene catheters were inserted into the esophagus and the pylorus. Saline was infused through the esophagus catheter with a constant infusion pump (Peristaltic Pump 132100, DESAGA, W.Germany) at the rate of 1 ml/min. Forty minutes after the start of perfusion, perfusate was collected from the pyloric catheter twice at 15-min intervals. Genipin and a gastric secretory stimulant were then administered into the duodenum and the femoral vein, respectively, and the perfusate was collected at 15-min intervals up to 75 min. The collected perfusate was titrated with 0.01N NaOH (phenolphthalein) to estimate acid concentration (mEq). Gastric secretory stimulants used were tetragastrin, carbachol, and Hist.

(2) Effect on Isolated Organs: i) Effect on Isolated Intestines: According to the Magnus cumulative method, effect of genipin was examined on contraction induced by ACh, Pilo, and 5-HT in the isolated mouse intestine and by Hist in the isolated guinea pig intestine. Tyrode solution was used and temperature of the organ bath (30 ml) was regulated to $27^\circ \pm 1$. ii) Effect on Isolated Fundus Strip Preparations: According to the method of Vane,³⁾ effect of genipin on contraction induced by ACh, 5-HT, and Hist in the rat fundus strip was examined. The fundus strip was suspended in Krebs solution (20 ml, $37^\circ \pm 1$) which was bubbled with a mixture of 95% O_2 and 5% CO_2 .

(3) Effect on Canalicular Bile Flow: Under

anesthesia with pentobarbital sodium (50 mg/kg, *i.p.*), rats weighing about 230 g were laparotomized, and both ureters were tied. ^{14}C -labeled erythritol of 4 μCi (1 $\mu\text{Ci}/0.1$ ml saline) was administered as a single intravenous injection (*i.v.*). A 2-hr period was then allowed for equilibration, after which the common bile duct was cannulated with a polyethylene catheter, and the bile was drained out for 10 min. The test chemical solution was then administered in the duodenum (*i.d.*) and again the bile was drained for 5 min, after which the bile was collected for 10 min and weighed; no correction was made for specific gravity. Blood samples were taken from the carotid artery at the endpoint of the bile collection period. ^{14}C -erythritol activity in the bile and plasma was measured using scintillation medium, Aquasol-2 (New England Nuclear, U.S.A., Daiichikagaku Yakuhin, Tokyo), in a liquid scintillation counter (Aloka Model LSC-602). The biliary clearance was calculated from the formula: $\text{dpm bile/dpm plasma} \times \mu\text{l/min/kg b.w.}$ Sodium dehydrocholate (DHC) was used as a standard.

(4) Effect on Biliary Components: a) Bile Flow, Solid Weight of the Bile, Bile Acids, Cholesterol, and Phospholipids: Under anesthesia with pentobarbital sodium (50 mg/kg, *i.p.*), rats weighing 200–250 g were laparotomized, a polyethylene cannula was inserted into the common bile duct, and the bile was collected for 1 hr. The test chemical solution was then administered into the duodenum and the bile was collected at 1-hr intervals to measure the biliary components, bile flow, solid weight of bile, and concentration of bile acids, cholesterol, or phospholipids in the bile. In order to investigate the relationship between bile flow and bile acid concentration, the rat was surgically operated and the test chemical solution was administered *i.d.* as above, and the bile was then collected at 15-min intervals to measure the volume and the concentration of bile acids. The bile was collected during the first 5-min period beginning 10 min after administration of the test chemical solution, and the volume of bile and the concentration of bile acids were measured. b)

TABLE I. Effect of Genipin on Gastric Acid Output induced by Carbachol, Tetragastrin, and Histamine in Rats

Stimulants (i.v.)	Compounds mg/kg (i.d.)	No. of rats	Acid output ($\mu\text{Eq}/15\text{min}$)						
			-30	-15	Administration ^{a)}				
					15	30	45	60	75 min
Carbachol 5 $\mu\text{g}/\text{kg}$	Control	— 14	3.7 $\pm$ 0.4 ^{b)}	3.5 $\pm$ 0.4	7.4 $\pm$ 0.7	15.6 $\pm$ 0.8	10.9 $\pm$ 1.0	8.0 $\pm$ 1.0	5.7 $\pm$ 0.5
	Genipin	25 10	3.3 $\pm$ 0.3	3.3 $\pm$ 0.3	6.9 $\pm$ 0.7	12.5 $\pm$ 1.4	10.4 $\pm$ 0.1	6.3 $\pm$ 0.5	5.2 $\pm$ 0.4
	Genipin	50 14	3.0 $\pm$ 0.2	2.9 $\pm$ 0.2	5.7 $\pm$ 0.4 [*]	11.1 $\pm$ 0.9 [*]	8.5 $\pm$ 0.6 [*]	5.8 $\pm$ 0.4	4.5 $\pm$ 0.4
	Atropine	1 10	3.5 $\pm$ 0.3	3.5 $\pm$ 0.3	3.8 $\pm$ 0.3 ^{***}	3.3 $\pm$ 0.2 ^{***}	2.8 $\pm$ 0.3 ^{***}	2.4 $\pm$ 0.2 ^{***}	2.2 $\pm$ 0.2 ^{***}
Tetragastrin 15 $\mu\text{g}/\text{kg}$	Control	— 13	4.8 $\pm$ 0.5	3.9 $\pm$ 0.4	6.2 $\pm$ 0.6	7.5 $\pm$ 0.9	5.0 $\pm$ 0.6	4.0 $\pm$ 0.4	3.4 $\pm$ 0.4
	Genipin	50 11	4.8 $\pm$ 0.9	5.1 $\pm$ 1.1	5.5 $\pm$ 0.8	7.8 $\pm$ 1.2	5.3 $\pm$ 0.8	4.0 $\pm$ 0.3	3.8 $\pm$ 0.4
Histamine 0.5 mg/kg	Control	— 14	3.3 $\pm$ 0.5	3.1 $\pm$ 0.4	4.3 $\pm$ 0.6	6.1 $\pm$ 0.9	5.4 $\pm$ 0.8	3.9 $\pm$ 0.5	3.3 $\pm$ 0.4
	Genipin	50 9	4.2 $\pm$ 0.5	4.1 $\pm$ 0.4	4.5 $\pm$ 0.5	6.8 $\pm$ 0.7	6.5 $\pm$ 0.8	4.7 $\pm$ 0.4	4.7 $\pm$ 0.4
Histamine 1 mg/kg	Control	— 11	3.8 $\pm$ 0.5	3.5 $\pm$ 0.5	4.3 $\pm$ 0.4	7.9 $\pm$ 0.8	7.3 $\pm$ 1.0	5.7 $\pm$ 0.8	4.9 $\pm$ 0.9
	Genipin	50 11	3.5 $\pm$ 0.3	3.2 $\pm$ 0.3	4.4 $\pm$ 0.3	7.1 $\pm$ 0.7	5.9 $\pm$ 0.6	4.4 $\pm$ 0.4	3.4 $\pm$ 0.2
—	Genipin	50 13	3.9 $\pm$ 0.3	3.8 $\pm$ 0.3	3.5 $\pm$ 0.5	3.4 $\pm$ 0.4	3.2 $\pm$ 0.3	3.4 $\pm$ 0.5	3.7 $\pm$ 0.6

a) Stimulant: intravenous administration, compound: intraduodenal administration.

b) Mean $\pm$ S.E., *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Sodium, Potassium, Chloride, and Bicarbonate: Rats weighing 230–250 g were surgically operated as in a), the bile was drained for 30 min, and the test chemical solution was injected *i.d.*, after which the bile was collected at 30-min intervals. The ionic concentration, *i.e.*, Na^+ , K^+ , Cl^- , and HCO_3^- , was determined in each collected bile specimen. DHC was used as a standard. c) Analytical Methods: Solid Weight of the Bile: The collected bile was evaporated in a hot-air drier (Ishii Shoten Co.), thoroughly dried with a vacuum pump, and the dried product was weighed. Bile acids: Bile acids were determined by the enzymic method^{4–6)} using Sterognost-3 α (Nyegaard & Co. As. Oslo, Norway). Cholesterol: Bile (0.5 ml) was extracted three times with CH_2Cl_2 , the extract was evaporated and dried, and 1 ml of isopropanol was added to this residue. After shaking, 0.5 ml of this isopropanol solution was used as the test sample. Cholesterol was determined by the enzymic method using Determiner-TC (Kyowa Hakko Kogyo).

Recovery ratio of cholesterol and cholesteryl linolate by the present method was 90.6% and 96.4%, respectively. In the present experiments, the data are uncorrected. Phospholipids: Biliary

phospholipid concentration was measured by the enzymic method using Iatroset PL-E (OM) (Iatron Co.). Sodium, Potassium, Chloride, and Bicarbonate: Na^+ , K^+ , and Cl^- concentrations in the bile were measured by the ion selective electrode method⁷⁾ with Technicon Stat/Ion (Nihon Technicon Co.), and HCO_3^- content was measured by Natelson microgasometer⁸⁾ (Kayagaki Irikakogyo, Co., LTD).

RESULTS

1. Effect of Genipin on the Elevated Gastric Acid Secretion induced by Carbachol, Tetragastrin, and Histamine

After *i.v.* administration of carbachol (5 $\mu\text{g}/\text{kg}$), tetragastrin (15 $\mu\text{g}/\text{kg}$), and Hist (0.5–1 mg/kg), gastric acid secretion was accelerated immediately, reaching a peak after 30 min, and then decreased gradually to the initial level. Elevated gastric acid secretion was inhibited by intraduodenal (*i.d.*) administration of 25 mg/kg of genipin, and a significant inhibitory effect was observed during the period between 15 and 45 min after administration of 50 mg/kg of genipin. However, genipin showed no inhibitory effect on the gastric acid secretion induced by tetragastrin

FIG. 1. Effect of Genipin on the Dose Response Curve of the Contraction induced by Acetylcholine Chloride: A, Pilocarpine Hydrochloride: B, Histamine Dihydrochloride: C, and Serotonin Hydrochloride: D in the Isolated Mouse (A, B, and D) and Guinea Pig (C) Ileum

● — ●: control, ○ — ○: genipin 1×10^{-4} g/ml, ○ — — ○: genipin 5×10^{-4} g/ml, ○ — — — ○: genipin 1×10^{-3} g/ml, × — ×: a) atropine 1×10^{-9} g/ml; b) atropine 3×10^{-9} g/ml; c) diphenhydramine 1×10^{-8} g/ml; d) dibenamine 1×10^{-6} g/ml, ▲ — ▲: papaverine 5×10^{-6} g/ml. Numbers in the parentheses indicate the number of animals.

FIG. 2. Effect of Genipin on the Dose Response Curve of the Contraction induced by Acetylcholine Chloride: A, Serotonin Hydrochloride: B, and Histamine Dihydrochloride: C, in the Rat Stomach Fundus Strip. Numbers in the parentheses indicate the number of animals.

FIG. 3. *Choleretic Action of Genipin in Rats*
The bile was collected for 10 min from 5 min after the intraduodenal administration.
DHC: dehydrocholate-Na

and Hist. This result is shown in Table I.

2. Effect of Genipin on Isolated Organs

In the isolated intestines, 1×10^{-4} to 5×10^{-4} g/ml of genipin parallelly shifted the dose-response curve of the contraction induced by ACh and Pilo, although the parallelism in the latter case was not perfect (Fig. 1, A and B). The dose-response curve of Hist- and 5-HT-induced contraction was inhibited in the style of "bowed head" by 1×10^{-4} g/ml of genipin (Fig. 1, C and D). In the isolated fundus strip preparations, genipin showed an inhibitory effect in the

"bowed head" style on the dose-response curve of ACh- and 5-HT-induced contraction at 5×10^{-4} g/ml, and Hist-induced contraction at 5×10^{-5} g/ml (Fig. 2).

3. Effect of Genipin on the Canalicular Bile Flow

The bile flow increased significantly at the 10-min period from 5 min after *i.d.* administration of 50 mg/kg of genipin, and this effect was more intensive than that induced by DHC (Fig. 3). In this case, the bile-to-plasma concentration ratio of ^{14}C -erythritol was not changed significantly with genipin or DHC (Table II), and in the relationship between the bile flow and ^{14}C -erythritol clearance, genipin and DHC increased the clearance along with the bile flow (Fig. 4). In Fig. 4, equations of regression lines were: For control, $y = 1.034x - 3.60$ ($r = 0.98$); for genipin, $y = 0.907x + 2.00$ ($r = 0.98$); and for DHC, $y = 0.811x + 9.20$ ($r = 0.98$). For genipin and DHC, the slope of regression line was not significantly different from that of the control. In the case of the bile, bile sample from each animal was divided into 2 to 3 fractions, and ^{14}C -activity was determined. The value of the product of ^{14}C -activity of each fraction and the bile flow was averaged to give the value of ^{14}C -activity of the bile itself.

4. Effect of Genipin on the Amount of Solid Components, Bile Acids, Cholesterol, and Phospholipids in the Bile

Changes in bile flow and the amount of biliary components at 1-hr intervals after administration of genipin are shown in Fig. 5. In the experiment on bile collection at 1-hr intervals, genipin showed a significant facilitatory effect on bile secretion at 25 mg/kg and more effective at 50

TABLE II. *Effect of Genipin on Biliary ^{14}C -Erythritol Excretion in Rats*

Drug	Dose (mg/kg, <i>i.d.</i>)	No. of animals	Bile/Plasma concn. ratio of ^{14}C -erythritol
Control	—	17	0.957 ± 0.011^a
Genipin	50	15	0.929 ± 0.009
Dehydrocholate-Na	50	13	0.951 ± 0.012

a) Mean $\pm$ S.E.

FIG. 4. Relationship between ^{14}C (U)-Erythritol Clearance and Bile Flow after Intraduodenal Administration of Genipin in Rats

Equation of regression line is: for control, $y=1.034x-3.60$; for genipin, $y=0.907x+2.00$; for dehydrocholate-Na, $y=0.811x+9.20$.

FIG. 5. Effects of Genipin on the Flow and Composition of Bile in Rats

●—●: control ($n=10$), ○---○: genipin 25 mg/kg ($n=9$), ○—○: genipin 50 mg/kg ($n=10$), △—△: dehydrocholate-Na 50 mg/kg ($n=6$), ×—×: salicylate-Na 100 mg/kg ($n=7$).

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, i.d.: intraduodenal administration, Data are expressed as the mean $\pm$ S.E.

FIG. 6. Effect of Genipin on the Flow and Bile Acid Excretion of Bile in Rats

● — ● : control ($n=17$), ○ — ○ : genipin 50 mg/kg ($n=18$), △ — △ : dehydrocholate-Na 50 mg/kg ($n=11$), × — × : salicylate-Na 100 mg/kg ($n=10$).

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, i.d.: intraduodenal administration. Data are expressed as the mean $\pm$ S.E.

mg/kg 1 hr after *i.d.* administration. Genipin at 25–50 mg/kg did not affect the concentration of the biliary solid components at the peak period of the bile flow (1 hr), and the amount of solid components increased corresponding to increase in bile flow. On the other hand, the concentration of bile acids, cholesterol, and phospholipids in the bile decreased against bile secretion after administration of 25 and 50 mg/kg of genipin. DHC, 50 mg/kg, showed a choleric effect and increased the amount of biliary bile acids paralleling increase in the bile flow, whereas phospholipid concentration decreased at the same time.

5. Degree of Dependency of the Bile Acid on Genipin-induced Choleric Action

Changes in the flow, bile acid concentration, and bile acid excretion rate in the bile collected at 15-min intervals up to 60 min after *i.d.* administration of 50 mg/kg of genipin are shown in Fig. 6. Genipin in 50 mg/kg showed a significant choleric activity, reaching the peak 15 min after the administration, and the biliary bile acid concentration decreased inversely at this time. However, genipin did not affect the biliary bile acid excretion rate at every 15-min period. DHC showed an increase in the bile acid concentration and bile acid excretion rate in the bile accom-

panied with a choleric effect at 50 mg/kg (*i.d.*). Relationship between bile flow and bile acids in the bile for a period of 5 min beginning 10 min after administration of genipin (50 mg/kg, *i.d.*) is shown in Fig. 7. In this experiment, genipin showed a significant choleric effect by *i.d.* administration but the biliary bile acid excretion hardly changed. In contrast, increase in the biliary bile acid excretion was accompanied by a choleric effect with DHC. In both the control group and the test chemical-treated group, there was apparently linear relationship between bile flow and bile acid excretion rate, and the equation of each regression line was: For control, $y=0.0189x+0.027$ ($r=0.84$); for genipin, $y=0.0196x+0.079$ ($r=0.79$); for DHC, $y=0.0185x+0.033$ ($r=0.95$). Regression line of genipin was parallelly shifted upward against control and that of DHC was the same as that of the control.

6. Effect of Genipin on the Amount of Sodium, Potassium, Chloride, and Bicarbonate in the Bile

Changes in the biliary ionic concentration (Na^+ , K^+ , Cl^- , and HCO_3^-) at 15-min intervals after *i.d.* administration of 50 mg/kg of genipin are shown in Table III. Genipin did not show significant changes in each ionic concentration in the

FIG. 7 Relationship between Bile Acid Excretion Rate and Bile Flow after Intraduodenal Administration of Genipin and Dehydrocholate-Na in Rats.

Equation of regression line is: for control, $y = 0.0189x + 0.027$; for genipin, $y = 0.0196x + 0.079$; for dehydrocholate-Na, $y = 0.0185x + 0.033$.

bile against control. DHC at 50 mg/kg also showed no significant changes in the ionic concentration.

DISCUSSION

Genipin showed an inhibitory effect on the gastric acid secretion induced by carbachol, but not by tetragastrin, or histamine at doses of 25–50 mg/kg (*i.d.*). It is known that gastric secretion induced by carbachol is antagonized more effectively with atropine than that induced by tetragastrin or histamine.⁹⁾ Therefore, the result that genipin selectively antagonized carbachol-induced gastric secretion in the present study may be ascribed to the amount and/or potency of the drug administered. In the experiment on the contraction of isolated organs, genipin showed non-specific anti-ACh, anti-5-HT, and anti-Hist actions in the fundus strip preparation but competitive anti-ACh action in the intestine. Moreover, as reported previously,¹⁰⁾ genipin inhibited gastric acid secretion in pylorus-ligated rats. These results indicated that anticholinergic action at least partly contributed to the genipin-induced inhibitory effect on the gastric functions.

The bile acids in the bile are made up of the bile acids returning to hepatocytes after the

enterohepatic circulation^{11,12)} and the bile acid endogenously produced at hepatocytes by the exogenous bile acids.^{11,13)} The hepatic bile is secreted from hepatocytes into the canaliculi and also from biliary ductular cells. Concerning the former route, it is well known that the bile acids or substances composed of the bile acid-independent fraction such as Na^+ , K^+ , Cl^- , and HCO_3^- excreted independently of bile acids, were drained from hepatocytes into canaliculi by carrier-mediated active transport mechanism, osmotic gradient was created across the canalicular membrane, and then along its gradient the water originating in hepatocytes was transported into canaliculi. In the case of the latter route, it is also known that Na^+ , K^+ , Cl^- , and HCO_3^- were secreted into ducts and/or ductules under the effect of secretin and gastrin and, consequently, bile secretion was manifested.^{14,15)} As the reabsorptive function of biliary moisture to the ductular system was confirmed in dogs,^{14,16–22)} it seems that the bile secretion was increased or decreased according to inhibition or facilitation of the reabsorption of water into the ducts and/or ductules.

In the experiment on erythritol clearance, genipin (50 mg/kg, *i.d.*) significantly increased the flow for 10 min starting 5 min after administration, which was more effective than DHC,

TABLE III. *Effects on Biliary Ionic Concentration after intraduodenal (i.d.) Administration of Genipin in Rats*

Ion	Compound	Dose (mg/kg)	No. of animals	Ionic concentration (mM)						
				pre	30 ^{a)}	60	90	120	150	180 min
Na ⁺	Control	—	8	162.7	160.0	158.6	157.3	156.6	155.9	155.6
				±1.0	±1.3	±1.1	±1.0	±0.9	±0.9	±1.0
	Genipin	50	11	164.9	158.0	156.9	155.5	154.9	154.9	154.6
				±1.7	±0.8	±1.0	±1.0	±1.1	±1.2	±1.4
	Dehydrocholate-Na	50	8	164.1	158.8	158.7	158.3	157.7	157.7	156.5
				±0.8	±0.7	±0.8	±0.8	±0.6	±0.9	±1.1
K ⁺	Control	—	8	4.7	4.6	4.8	4.9	5.1	5.1	5.3
				±0.2	±0.1	±0.2	±0.1	±0.2	±0.2	±0.1
	Genipin	50	11	4.5	4.7	5.0	5.3	5.2	5.2	5.2
				±0.1	±0.1	±0.2	±0.2	±0.2	±0.2	±0.2
	Dehydrocholate-Na	50	8	4.8	5.0	4.9	5.1	5.2	5.3	5.4
				±0.1	±0.3	±0.1	±0.1	±0.2	±0.2	±0.2
Cl ⁻	Control	—	8	92.1	93.7	93.5	93.6	93.8	93.9	94.5
				±0.5	±0.6	±0.6	±0.6	±0.9	±0.8	±0.9
	Genipin	50	11	91.1	96.4	93.4	93.8	93.5	93.7	94.4
				±1.3	±3.2	±1.4	±1.3	±1.1	±1.2	±1.3
	Dehydrocholate-Na	50	8	92.6	90.5	91.2	90.9	92.0	93.2	94.8
				±1.2	±1.9	±1.6	±1.8	±1.5	±1.7	±1.6
HCO ₃ ⁻	Control	—	9	29.7	29.8	32.1	32.9	34.0	35.1	35.5
				±0.8	±0.9	±0.6	±1.0	±1.3	±2.6	±1.2
	Genipin	50	10	28.5	28.9	33.3	33.5	36.1	36.2	37.4
				5±0.9	±0.5	±1.1	±0.4	±0.8	±0.6	±1.2
	Dehydrocholate-Na	50	8	30.2	30.7	32.2	32.4	34.4	34.6	35.6
				±0.5	±0.4	±0.4	±0.6	±0.4	±0.6	±0.7

a) Time after administration.

with no change in the bile-to-plasma concentration ratio of erythritol. In the relationship between bile flow and erythritol clearance, there was no significant change between the equation of regression line obtained with genipin-treated group ($y=0.907x+2.0$, $r=0.98$) and with that of control ($y=1.034x-3.6$, $r=0.98$), and the two lines seemed identical. Based on these results, it was apparent that erythritol clearance was increased in accord with the increase in bile flow by the administration of genipin. Since Forker's experiment on erythritol clearance on the bile flow, many studies have been carried out,^{14, 16, 18, 19, 23-25} and from findings that bile acid-induced choleretic action was, but secretin-induced choleretic action was not,^{14, 16, 19} accompanied with the increase in erythritol clearance, it has

been accepted that the erythritol clearance theoretically expresses the canalicular bile flow.^{13, 14, 16} Therefore, in the present study, it was concluded that genipin-induced choleretic action was produced by the increase in canalicular bile formation. We used rats in the present study, but it has been reported that the rat was not sensitive enough to secretin to produce a choleretic action.^{19, 26}

As the facilitatory mechanism of canalicular bile formation, the followings are known: (1) Active transport of bile acids from hepatocytes into canaliculi, (2) increase in osmotic pressure by disruption of the bile acid micelles present in bile,^{13, 18, 19} (3) increase in osmotic pressure by the excretion of administered substances or their metabolites into the canalicular bile, (4) active trans-

port of bile acid-independent fraction from hepatocytes into canaliculi, and so on. Mechanism of (1) was not conceivable because the bile flow increased significantly after administration of genipin, but the biliary bile acid concentration decreased inversely, and the bile acid excretion rate was not changed.

Second, in the relationship between bile flow and bile acid excretion rate, the slope of regression line obtained from genipin-treated group was not significantly different from that of the control, and these lines were approximately in parallel. Since the slope of the regression line, which was calculated from plots of the bile flow to the bile acid excretion rate, indirectly expresses the osmotic pressure,¹⁸⁾ the slope of the control group (0.0189) obtained in the present study may show that 1 μ mol of bile acids creates about 19 mg of biliary moisture (bile flow). If the disruption of bile acid micelles occurred with genipin, 1 μ mol of bile acid should create more than 19 mg of water corresponding to a portion of disruption and theoretically the slope obtained from plots of the bile flow to the bile acid excretion rate should exceed that of the control. In this experiment, since the slope of regression line from genipin-treated group was 0.0196, which was not significantly different ($p > 0.05$) from that of control, it is difficult to accept the mechanism that genipin-induced choleretic action was caused by increased canalicular osmotic pressure due to disruption of bile acid micelles.

Third, although it was conceivable that canalicular osmotic pressure might be increased by excretion of genipin and genipin-derivatives into canaliculi, this mechanism was denied because, as described in a previous paper,²⁷⁾ when geniposide was administered into the portal vein, bile flow did not increase in spite of the excretion of far more geniposide into the bile than that by genipin and its glucuronide, both of which were excreted in the bile after intraportal administration of a choleretic dose of genipin.

Finally, in the relationship between bile flow and bile acid excretion rate, the difference between the equation of regression line from

genipin-treated group ($y = b_1x + a_1$) and that of control ($y = b_2x + a_2$) at $x = 0$, *i.e.*, $a_2 - a_1$ corresponded to the absolute increment in bile flow induced by genipin. The fact that, in the relationship between bile flow and bile acid excretion rate, the intersection of the regression line at the y axis is positive indicates that the bile will flow out continuously even if the bile acid excretion is lowered extremely. In bile fistula rats, the bile was flowing out although the concentration of bile acids was lowered below 2 μ mol/ml (below the critical micelle concentration) after the bile was drained out for several days.¹⁹⁾ In brief, the value of a_n in the equation expresses bile acid-independent fraction of canalicular origin. Genipin (50 mg/kg, *i.d.*) did not affect the concentration of Na^+ , K^+ , Cl^- or HCO_3^- in the bile collected during the first 30-min interval after administration. This result indicates that the amount of electrolytes excreted increased in accord with the increment of bile flow after administration of genipin. As Na^+ concentration was the greatest among them, it is conceivable that the role of Na^+ on the bile secretion is very important. Main components of the bile acid-independent fraction are electrolytes, therefore, it is concluded that genipin-induced choleretic action proceeds by the mechanism wherein water is driven along osmotic gradient which originates in the transport of bile acid-independent fraction from hepatocytes into canaliculi, mainly through active Na^+ transport.

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