STUDIES ON ENEMA ON THE ABSORPTION OF ISONICOTINIC ACID HYDRAZIDE IN LARGE INTESTINE*

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INTRODUCTION

Previously, the author (1) recognized, in the administration of streptomycin alone as enema or subcutaneous injection in the rabbits, that the blood streptomycin level reaches the maximum at 30 minutes and becomes trace or disappears at 6 hours after the drug alone was administered as enema. However, it was also known that, in the subcutaneous injection of this drug alone, the level reaches the highest value at 1 hour after the administration showing much higher value and much longer period of duration. After the above experiments, as the results of the administration of streptomycin combined with various substances as enema, the following facts were known. When streptomycin was administered as enema in combination with urethane, the blood streptomycin level reaches the maximum at 30 minutes after the administration, and it is proved to be present even after 6 hours, while magnesium sulfate does not show any particular influence on the level. When combined with chloral hydrate, the maximum value is reached at 30 minutes, and the presence of the drug is proved even after more than 6 hours. The blood streptomycin level in this case was also extremely high. Similar results were obtained when streptomycin was administered in combination with the isotonic solution of glucose and taurin together with chloral hydrate. In this case, the highest value was reached at 30 minutes and a high level was maintained even at 1-2 hours after the administration. Even after 6 hours, the level was found to be as high as it could be quantitatively estimated. However, in view of the fact that no clear effect on the absorption of streptomycin was noted in the case of the combination with taurin and glucose, the streptomycin absorption promoting effect of chloral hydrate was considered to be great. Furthermore, cattle bile and spreading factor were tried out in combination with streptomycin, and it was found that no particular effect by Sprase on the absorption of streptomycin in large intestine is exercised. On the other hand, the streptomycin absorption promoting effect of cattle bile was found extremely remarkable, showing a very high blood streptomycin level with a long duration time,

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Isonicotinic acid hydrazide (INAH hereafter) was discovered to be a powerful anti-tuberculosis preparation by J. Bernstein, W.A. Lott, B.A. Steinberg and H.L. Yale (2) in 1952, and its pronounced effect in the experimental animal tuberculosis as well as in the human tuberculosis cases was published. Since then, the results of the fundamental as well as clinical investigations have been reported by numerous workers (3-5). As the results of these reports, it was made clear that INAH is absorbed and excreted rapidly in the animal body, and that it shows a clear effect on the human tuberculosis, but that it develops various side reactions if the dosage is not adequate (6, 7).

In this instance, the author attempted to investigate the absorption of INAH in large intestine similarly to the case of streptomycin. In addition, various narcotics, bile, amino acids, urea, surface active agents and other substances were employed in combination with INAH to investigate the influences of these substances on the absorption of INAH in large intestine.

MATERIALS AND METHODS

Healthy mature white rabbits weighing approximately 2 kg were employed as experimental animals; they were not fed at all during the period of experiment.

The animals were bled by heart puncture; quantitative estimation of INAH was made according to the colorimetric method by J.M. Kelly, R.B. Poet *et al.* (8). The estimations were conducted at 30 minutes, 1 hour, 3 hours and 6 hours after the administration.

The administration of these substances into large intestine was made as enema according to the previous papers published by the workers of the author's Department. Each dose of the enema was 10 cc per kg of the animal, and before administration the enema was warmed up to the body temperature in all instances.

The preparation of INAH employed in this experiment was TUBERIAN (Bayer). The substances administered in combination with INAH were chloral hydrate, phenobarbital, urethane, magnesium sulfate, cattle bile, taurin, glycocoll, histidine, urea, thiourea, glucose and Sprase. Cattle bile was collected from the gall bladder within several hours from the slaughtering, and was sterilized and stored for experiments. The surface active agents employed were Emulgen 106 (dodecylalcohol polyoxyethylene ether), Hyren (sodium lauryl sulfate) and Tween 20 (polyoxyethylene ether of sorbitan monolaurate).

RESULTS

I. Enema of INAH alone (Table 1)

1) Administration of 25 mg of INAH

At the room temperature of 14°-18°C, the rabbits, Nos. 101-104, weighing approximately 2 kg, were given 25 mg of INAH as enema. In most cases, the blood plasma INAH level began to rise immediately, and 30 minutes after the administration, it reached the maximum (2.95-4.25 γ /cc). After 1 hour, it dropped slightly showing value of 2.40-

3.85 γ /cc, and after 3 hours, it dropped to 1.40–2.58 γ /cc, which is approximately 50% of the values after 30 minutes from the administration. Thereafter, it diminished gradually to reach 0.35–0.85 γ /cc at 6 hours from the administration.

2) Administration of 50 mg of INAH

In the case with doubled dose of INAH at the room temperature of 14°–18°C, the blood plasma INAH level in the animals (Rabbit Nos. 105–107) was approximately 2.5 times as high as that with 25 mg of INAH showing the maximum value of 8.75–9.95 γ /cc at 30 minutes after the administration. It maintained a relatively high level of 6.80–8.50 γ /cc even after 1 hour from the administration. It diminished thereafter to reach 1.85–2.85 γ /cc at 6 hours from the administration.

3) Administration of 100 mg of INAH

When the dose was further doubled to 100 mg, the blood plasma level of the rabbits (Nos. 108–110) was found remarkably high. It reached the maximum value of 12.50–14.00 γ /cc at 30 minutes after the administration, and although the value gradually dropped, it maintained a relatively high value of 7.50–9.85 γ /cc even at 3 hours after the administration. The average value after 6 hours was 4.15 γ /cc, which is less than 30% of the value at 30 minutes after the administration. Two out of three rabbits employed looked restless since approximately 45 minutes after the administration.

TABLE I. Blood INAH levels in rabbits after clyster with INAH alone

Rabbit No. Sex	Weight (kg)	Substances administered and dosage	Blood INAH level after (γ /cc)			
			30min	1hr	3hr	6hr
No. 101 ♀	2.1	INAH 25 mg	3.70	3.85	1.75	0.80
No. 102 ♂	2.3	"	4.25	3.50	2.58	0.65
No. 103 ♀	2.0	"	2.95	2.40	1.40	0.35
No. 104 ♂	2.2	"	3.85	3.40	1.65	0.85
No. 105 ♂	1.8	INAH 50 mg	9.95	6.80	4.25	1.85
No. 106 ♀	2.1	"	8.75	7.50	6.30	2.50
No. 107 ♂	2.1	"	9.50	8.50	7.00	2.85
No. 108 ♂	1.9	INAH 100 mg	13.00	12.50	9.85	5.35
No. 109 ♂	1.8	"	14.00	12.50	8.20	3.50
No. 110 ♀	2.0	"	12.50	11.45	7.50	3.60

TABLE 2. Blood INAH levels in rabbits after rectal INAH in the combination with chloral hydrate, phenobarbital, urethane or magnesium sulfate

Rabbit No. Sex	Weight (kg)	Substances administered and dosage	Blood INAH level after (γ/cc)			
			30min	1hr	3hr	6hr
No. 111 ♀	2.3	INAH 25 mg Chloral hydrate 0.3 g/kg	6.15	5.05	1.87	1.03
No. 112 ♂	2.0	"	4.85	3.25	1.65	0.75
No. 113 ♀	2.1	INAH 25 mg Chloral hydrate 0.5 g/kg	5.58	5.10	1.55	0.78
No. 114 ♂	2.2	"	7.00	4.56	1.65	1.25
No. 115 ♂	2.1	INAH 25 mg Chloral hydrate 0.7 g/kg	11.25			
No. 116 ♂	1.7	"	7.30	7.80		
No. 117 ♀	2.0	INAH 25 mg Phenobarbital 0.3 g/kg	3.75	4.50	5.00	
No. 118 ♂	1.7	"	3.00	3.60	4.05	
No. 119 ♂	2.0	INAH 25 mg Phenobarbital 0.5 g/kg	6.18			
No. 120 ♀	1.9	"	7.00			
No. 121 ♀	2.2	INAH 25 mg Urethane 1.0 g/kg	4.58	3.40	2.75	0.90
No. 122 ♀	1.9	"	5.60	5.00	3.10	1.50
No. 123 ♀	2.0	INAH 25 mg Magnesium sulfate 1.0 g/kg	3.85	3.54	3.00	0.75
No. 124 ♂	2.1	"	3.50	3.50	2.24	1.00

II. The influence of chloral hydrate, phenobarbital, urethane or magnesium sulfate on the absorption of INAH in large intestine (Table 2)

1) Enema of INAH combined with chloral hydrate

When two rabbits (Nos. 111 and 112) weighing approximately 2 kg were given 25 mg each of INAH combined with 0.3 g per kg of chloral hydrate, only slightly slowed respiration rate without remarkable changes in the reflex was noted, and the anaesthesia passed mildly. The blood plasma INAH level was found higher than observed when 25 mg of INAH alone was administered. The level reached the maximum (4.85 γ /cc and 6.15 γ /cc respectively) at 30 minutes after the administration. It gradually diminished thereafter to drop to 0.75 γ /cc and 1.03 γ /cc respectively at 6 hours after the administration.

When the amount of chloral hydrate was increased to 0.5 g per kg, the rabbits (Nos. 113 and 114) demonstrated slow respiration rate, disappearance of reflex and lethargic state as early as at 30 minutes after the administration, and recovered from them at 6 hours after the administration. In this case, blood plasma INAH level was high during the period between 30 minutes and 1 hour after the administration, showing the highest value (5.58 γ /cc and 7.00 γ /cc respectively) at 30 minutes after the administration. A relatively high level was maintained even after 1 hour. Although the level gradually diminished thereafter, the level at 3 hours after the administration was found not much different from that in the case with 25 mg of INAH alone, and the level at 6 hours was 0.78 γ /cc and 1.25 γ /cc respectively.

When two rabbits (Nos. 115 and 116) were treated with further increased amount of chloral hydrate (0.7 g per kg), all of the animals demonstrated slow respiration rate, disappearance of reflex, lethargy and collapse at 30 minutes after the administration, and became fatal at 40 minutes and 80 minutes respectively after the administration. In this case, the blood plasma INAH level was found extremely high. In the case of the rabbit No. 115, the level after 30 minutes was 11.25 γ /cc, and in the case of the rabbit No. 116, the level after 30 minutes and 1 hour was 7.30 γ /cc and 7.80 γ /cc respectively, showing approximately twice as high as that in the cases with 25 mg of INAH alone.

2) Enema of INAH combined with phenobarbital

When two rabbits, Nos. 117 and 118, were treated with 25 mg of INAH combined with 0.3 g per kg of phenobarbital, all of the animals demonstrated disappearance of reflex and were found lying on the side and collapsed as early as at 30 minutes; they were lethargic at 1 hour, and died after 3 hours and 6 hours respectively. In this case, the blood plasma INAH level was approximately as high as that in the cases with 25 mg of INAH alone after 30 minutes, but it rose gradually thereafter to reach the highest value of 4.05 γ /cc and 5.00 γ /cc respectively after 3 hours.

When the amount of phenobarbital was increased to 0.5 g per kg, rabbits (Nos. 119 and 120) became lethargic as early as at 30 minutes after the administration, and died within 1 hour after the administration. The blood plasma INAH levels in these cases

were 6.18 γ /cc and 7.00 γ /cc respectively, which are evidently higher than those recognized in the cases with 25 mg of INAH alone.

3) *Enema of INAH combined with urethane*

When two rabbits, Nos. 121 and 122, were treated with 25 mg of INAH combined with 1.0 g per kg of urethane, the rabbits demonstrated diminished reflex and collapse at 30 minutes after the administration, but the reflex was found moderately recovered and became able to move around though sluggishly after 3 hours. The maximum level was reached after 30 minutes, but it was almost equal to those observed in the cases with 25 mg of INAH alone. However, the level after 3 hours was found relatively high showing 2.75 γ /cc and 3.10 γ /cc respectively, and, after 6 hours, it was 0.90 γ /cc and 1.50 γ /cc respectively.

4) *Enema of INAH combined with magnesium sulfate*

When two rabbits, Nos. 123 and 124, were treated with 25 mg of INAH combined with 1.0 g per kg of magnesium sulfate, the animals showed no symptoms. The blood plasma INAH level reached the highest value (3.50 γ /cc and 3.85 γ /cc respectively) after 30 minutes, which are approximately equal to those observed in the cases with 25 mg of INAH alone. However, the level was found slightly higher than those of the latter cases after 3 hours.

III. *The influence of cattle bile, taurin, glycoll or histidine on the absorption of INAH in large intestine (Table 3)*

1) *Enema of INAH combined with cattle bile*

When two rabbits, Nos. 131 and 132, weighing approximately 2 kg, were given 25 mg of INAH combined with 0.5 g per kg of cattle bile, the animals demonstrated no changes in the general condition. The blood plasma INAH level was found slightly higher than those noted in the cases with 25 mg of INAH alone. At 30 minutes after the administration, the highest value (4.35 γ /cc and 5.25 γ /cc respectively) was reached: the value after 1 hour was 4.25 γ /cc and 4.50 γ /cc respectively. It gradually diminished to a lower value, 2.05–2.25 γ /cc and 0.86–0.90 γ /cc respectively, after 3 hours and 6 hours.

When the amount of cattle bile was increased to 1.0 g per kg, the animals (Rabbit Nos. 133 and 134) became sluggish in the movement of the whole body at 30 minutes after the administration. The blood plasma INAH level in this case was found higher compared with the above case, and the level was maintained for relatively longer period of time. The level reached the highest value (5.35 γ /cc and 5.36 γ /cc respectively) at 30 minutes after the administration; the level was relatively high (2.30 γ /cc and 3.50 γ /cc respectively) even after 3 hours. It gradually diminished thereafter to show 1.00 γ /cc and 1.25 γ /cc respectively after 6 hours from the administration, indicating higher values than that after 6 hours in the case with INAH alone.

2) *Enema of INAH combined with taurin*

When two rabbits, Nos. 135 and 136, were given 25 mg of INAH combined with 0.5 g per kg of taurin, the animals did not show any changes in the general condition. The highest blood plasma INAH level was found either after 30 minutes (3.46 γ /cc and 4.80 γ /cc respectively) or after 1 hour (3.85 γ /cc and 4.43 γ /cc respectively). A gradual diminution in the level was observed thereafter, and 3-6 hours after the administration the level was almost equal to that in the case with 25 mg of INAH alone.

TABLE 3. Blood INAH levels in rabbits after rectal INAH in the combination with cattle bile, taurin, glycocoll or histidine

Rabbit No. Sex	Weight (kg)	Substances administered and dosage	Blood INAH level after (γ /cc)			
			30min	1hr	3hr	6hr
No. 131 ♀	1.8	INAH 25 mg Cattle bile 0.5 g/kg	5.25	4.50	2.05	0.86
No. 132 ♀	2.1	"	4.35	4.25	2.25	0.90
No. 133 ♂	2.2	INAH 25 mg Cattle bile 1.0 g/kg	5.35	4.35	3.50	1.25
No. 134 ♀	1.7	"	5.36	3.85	2.30	1.00
No. 135 ♂	2.0	INAH 25 mg Taurin 0.5 g/kg	3.46	3.85	1.20	0.70
No. 136 ♂	1.8	"	4.80	4.43	2.12	0.53
No. 137 ♂	1.9	INAH 25 mg Glycocoll 0.5 g/kg	4.25	4.50	2.95	1.40
No. 138 ♀	2.0	"	3.75	4.25	1.40	0.75
No. 139 ♂	1.9	INAH 25 mg Histidine 0.5 g/kg	3.24	4.60	2.28	0.78
No. 140 ♀	1.8	"	4.20	4.00	2.13	1.30

3) *Enema of INAH combined with glycocoll*

No particular changes were observed when two rabbits, Nos. 137 and 138, were given 25 mg of INAH combined with 0.5 g per kg of glycocoll. The blood plasma INAH level rose at 30 minutes after the administration, and the highest level (4.25 γ /cc and 4.50 γ /cc respectively) was reached after 1 hour. A tendency of gradual diminution in the level thereafter was noted showing almost an equal level as was noted in the case with 25 mg of INAH alone.

4) *Enema of INAH combined with histidine*

When 0.5 g per kg of histidine was given in combination with INAH, neither of the animals showed any changes, showing the highest blood plasma INAH level during

the period at 30 minutes and 1 hour after the administration. In the case of rabbit No. 139, the highest level, 4.60 γ /cc, was reached at 1 hour after the administration, and in the case of rabbit No. 140, the highest level, 4.20 γ /cc, was reached at 30 minutes after the administration. In both of the cases, a gradual diminution in the level was noted showing a lower level of 0.78 γ /cc and 1.30 γ /cc respectively after 6 hours. When these values were compared with those in the case with 25 mg of INAH alone, no remarkable difference was recognized.

All the above experiments were performed at the room temperature of 14°-18°C.

IV. The influence of urea, thiourea, glucose or Sprase on the absorption of INAH in large intestine (Table 4)

1) Enema of INAH combined with urea

When two rabbits, Nos. 141 and 142, weighing approximately 2 kg, were given 25 mg of INAH combined with 3.0 g per kg of urea, no remarkable changes excepting sluggish movement was demonstrated by either of the animals. The blood plasma INAH level was found relatively low, showing the highest level (2.88 γ /cc and 3.03 γ /cc-2.88 γ /cc and 2.95 γ /cc respectively) during the period at 30 minutes and 1 hour after the administration, which gradually diminished thereafter as the time elapsed.

2) Enema of INAH combined with thiourea

When two rabbits, Nos. 143 and 144, were given 25 mg of INAH combined with 3.0 g per kg of thiourea, the animals were found lying on the side occasionally and

TABLE 4. Blood INAH levels in rabbits after rectal INAH in the combination with urea, thiourea, glucose or Sprase

Rabbit No. Sex	Weight (kg)	Substances administered and dosage	Blood INAH level after (γ /cc)			
			30min	1hr	3hr	6hr
No. 141 ♂	1.9	INAH 25 mg Urea 3.0 g/kg	3.03	2.95	1.80	0.88
No. 142 ♂	1.8	"	2.88	2.88	1.60	0.90
No. 143 ♂	2.3	INAH 25 mg Thiourea 3.0 g/kg	3.35	3.85	2.95	1.20
No. 144 ♂	2.0	"	2.85	2.75	1.75	0.75
No. 145 ♂	2.2	INAH 25 mg Glucose 3.0 g/kg	2.73	2.66	1.50	1.15
No. 146 ♂	1.9	"	2.88	2.50	1.75	0.65
No. 147 ♀	1.8	INAH 25 mg Sprase 5000 v.u./kg	6.25	5.35	4.00	1.05
No. 148 ♀	2.1	"	6.85	4.90	3.13	0.75

sluggish movement was recognized at 1 hour after the administration. The blood plasma INAH level in this instance was somehow equal to that in the case with 25 mg of INAH alone, showing the highest level (2.85 γ /cc and 3.35 γ /cc-2.75 γ /cc and 3.85 γ /cc respectively) during the period at 30 minutes and 1 hour after the administration. The level after 3 hours was 1.75 γ /cc and 2.95 γ /cc respectively, which diminished gradually thereafter.

3) *Enema of INAH combined with glucose*

When the enema of INAH combined with 3.0 g per kg of glucose was given to the rabbits as enema, no intoxication symptoms were noted in the animals. The blood plasma INAH level was found to be generally lower, showing the highest level (2.73 γ /cc and 2.88 γ /cc respectively) at 30 minutes after the administration. The level gradually diminished thereafter showing the value of 1.50 γ /cc and 1.75 γ /cc respectively after 3 hours.

4) *Enema of INAH combined with Sprase*

In the case when 25 mg of INAH combined with 5,000 v.u.m. per kg of Sprase, no particular changes in the general condition of the rabbits, Nos. 147 and 148, were noted. However, the blood plasma INAH level was found evidently high, showing the highest value (6.25 γ /cc and 6.85 γ /cc respectively) at 30 minutes after the administration. Moreover, the duration of the high level was also found long, showing the level of 3.13 γ /cc and 4.00 γ /cc respectively, even after 3 hours. The value diminished thereafter showing 0.75 γ /cc and 1.05 γ /cc respectively after 6 hours.

All of the above experiments were performed at the room temperature of 14°-18°C.

V. *The influence of surface active agents on the absorption of INAH in large intestine*

1) *Enema of INAH combined with Emulgen (Table 5)*

When the enema of 25 mg of INAH combined with 0.25 g per kg of Emulgen 106 was given to two rabbits, Nos. 151 and 152, no remarkable changes in the animals were noted. The blood plasma INAH level in this case was found evidently higher and was maintained longer compared with that in the case with 25 mg of INAH alone. The highest level (6.00 γ /cc and 7.25 γ /cc respectively) was reached after 30 minutes from the administration, which gradually diminished thereafter to 2.03 γ /cc and 2.25 γ /cc respectively after 3 hours, and to 1.22 γ /cc and 1.35 γ /cc respectively after 6 hours.

When the amount of Emulgen 106 was increased to 0.5 g per kg in the case of the rabbit No. 153, a slight rise in the respiration rate was noted, but no remarkable change was noted in the case of the rabbit No. 154. The blood plasma INAH level in this case was found slightly higher than that in the former experiment. The highest value was reached during the period at 30 minutes and 1 hour after the administration in both of the rabbits. In the case of No. 153, the value was 8.76 γ /cc at 30 minutes and 8.65 γ /cc at 1 hour, and in the case of No. 154, 6.25 γ /cc at 30 minutes and 6.80 γ /cc at 1 hour. At 3 hours after the administration, though the value was found diminished in both of the cases, it was 2.75 γ /cc and 3.50 γ /cc. The value was 1.30 γ /cc and 2.50 γ /cc respectively at 6 hours after the administration.

TABLE 5. Blood INAH levels in rabbits after rectal INAH in the combination with Emulgen 106

Rabbit No. Sex	Weight (kg)	Substances administered and dosage	Blood INAH level after (γ /cc)			
			30min	1hr	3hr	6hr
No. 151 ♂	1.8	INAH 25 mg Emulgen 0.25 g/kg	7.25	5.36	2.25	1.35
No. 152 ♂	1.8	"	6.00	5.75	2.03	1.22
No. 153 ♀	2.2	INAH 25 mg Emulgen 0.5 g/kg	8.76	8.65	3.50	2.50
No. 154 ♂	2.0	"	6.25	6.80	2.75	1.30
No. 155 ♀	1.8	INAH 25 mg Emulgen 1.0 g/kg	8.45	8.45	5.36	2.28
No. 156 ♂	1.9	"	6.25	5.00	2.75	1.55

In the case when the amount of Emulgen 106 was further increased to 1.0 g per kg, no remarkable difference in the blood plasma INAH level was noted compared with that in the former experiments, showing the highest value (6.25 γ /cc and 8.45 γ /cc respectively) at 30 minutes after the administration. The level was found relatively high even after 3 hours, and it was 1.55 γ /cc and 2.28 γ /cc respectively after 6 hours.

2) Enema of INAH combined with Hyren (Table 6)

When rabbits were given enema of 25 mg of INAH combined with 0.25 g per kg of Hyren, no particularly remarkable changes were noted in the animals. The blood plasma INAH level in this case was much higher and was maintained longer compared with that in the case with 25 mg of INAH alone. In the case of No. 157, the level at 30 minutes and 1 hour after the administration was 9.25 γ /cc and 8.80 γ /cc respectively, and in the case of No. 158, it was 7.63 γ /cc and 8.00 γ /cc respectively. Even after 3 hours, the level in both of the cases was as high as 6.10 γ /cc and 4.10 γ /cc respectively. In spite of the gradual diminution thereafter, the value was 4.25 γ /cc and 2.75 γ /cc respectively after 6 hours, showing a process at a clearly high concentration.

When the amount of Hyren was increased to 0.5 g per kg (Rabbit Nos. 159 and 160), the blood plasma INAH was lower compared with that in the above case, but evidently higher than that in the case with 25 mg of INAH alone. The level was the highest (6.00 γ /cc and 6.35 γ /cc) at 30 minutes after the administration, 5.00 γ /cc and 5.51 γ /cc at 1 hour after, and was 3.52 γ /cc and 5.25 γ /cc respectively at 3 hours after. Both of the rabbits died with moderate intoxication 3-6 hours after the administration.

When the amount of Hyren was increased to 1.0 g per kg, the rabbits, Nos. 161 and 162, demonstrated dyspnea and arrhythmic heart beat as early as at 15 minutes after the administration and died after 50 minutes and 90 minutes respectively. The blood plasma

INAH level in this case was not much different from that in the case with 25 mg of INAH combined with 0.5 g per kg of Hyren, showing 6.25 γ /cc and 6.00 γ /cc respectively at 30 minutes after the administration.

3) *Enema of INAH combined with Tween 20 (Table 6)*

When 25 mg of INAH combined with 0.25 g per kg of Tween 20 was given to the rabbits, Nos. 163 and 164, no particular changes were noted in the animals. A clear rise of the blood plasma INAH level was noted during the period from 30 minutes to 3 hours after the administration, but the blood plasma INAH level at 6 hours after the administration was not much different from that in the case with 25 mg of INAH alone. It was 6.76 γ /cc and 7.60 γ /cc respectively after 30 minutes, and was 5.40 γ /cc and 6.70 γ /cc respectively after 1 hour showing a slight diminution. It was 2.75 γ /cc and 3.50 γ /cc respectively after 3 hours and was 1.00 γ /cc and 1.25 γ /cc respectively after 6 hours.

When the amount of Tween 20 was increased to 0.5 g per kg in the cases of the rabbits, Nos. 167 and 168, only a slight increase in the number of heart beat was noted in both of the animals since 30 minutes after the administration. The blood plasma INAH level demonstrated roughly the same tendency as seen in the former experiments, showing the highest value (6.50 γ /cc and 7.30 γ /cc respectively) after 30 minutes. The value indicated a tendency of gradual diminution thereafter.

TABLE 6. Blood INAH levels in rabbits after rectal INAH in the combination with Hyren or Tween 20

Rabbit No. Sex	Weight (kg)	Substances administered and dosage	Blood INAH level after (γ /cc)			
			30min	1hr	3hr	6hr
No. 157 ♂	1.8	INAH 25 mg Hyren 0.25 g/kg	9.25	8.80	6.10	4.25
No. 158 ♀	2.1	"	7.63	8.00	4.10	2.75
No. 159 ♀	2.0	INAH 25 mg Hyren 0.5 g/kg	6.00	5.51	3.52	
No. 160 ♀	2.0	"	6.35	5.00	5.25	
No. 161 ♂	2.1	INAH 25 mg Hyren 1.0 g/kg	6.25	6.25		
No. 162 ♀	2.2	"	6.00			
No. 163 ♀	2.2	INAH 25 mg Tween 0.25 g/kg	6.76	5.40	2.75	1.00
No. 164 ♂	1.8	"	7.60	6.70	3.50	1.25
No. 167 ♀	2.1	INAH 25 mg Tween 0.5 g/kg	7.30	5.83	3.40	1.70
No. 168 ♀	2.2	"	6.50	5.50	3.00	1.35

SUMMARY

When the absorption of INAH in large intestine was investigated, various narcotics, cattle bile, amino acids, urea, glucose, various spreading factors and surface active agents were employed in combination with INAH as enema in an attempt to investigate the influence of the above substances on the absorption of INAH in large intestine.

Prior to the above investigation, 25 mg, 50 mg and 100 mg of INAH alone were given respectively as enema to the rabbits weighing approximately 2 kg, and the blood plasma INAH level of these animals was estimated. In the cases with 25 mg, the highest level was attained at 30 minutes after the administration and the level gradually diminished as the time elapsed. In the case with 50 mg, the highest level was attained also at 30 minutes after the administration, and the level gradually diminished as the time elapsed. However, in these cases, the blood plasma INAH level was approximately 2.5 times as high as that in the preceding cases was maintained. When the amount of INAH was doubled to reach 100 mg, the highest level was attained at 30 minutes after the administration, and the level gradually diminished to reach less than 30% of that at 6 hours after the administration. However, this value was found to be slightly higher than that at 30 minutes with 25 mg of INAH. Particularly in the cases last mentioned, in 2 out of 3 rabbits, restlessness was noted since 45 minutes after the administration, though they did not become fatal.

In the cases when other substances were combined with INAH, the dose of INAH was fixed to 25 mg. When 0.3 g and 0.5 g per kg of chloral hydrate was given in combination with INAH, in both of the cases the blood plasma INAH level at 30 minutes-1 hour was found to be slightly but evidently higher. Further increase in the amount of chloral hydrate to 0.7 g per kg remarkably promoted the absorption of INAH, but the rabbits died at 40 minutes and 2 hours respectively after the administration with intoxication symptoms. When 0.3 g per kg of phenobarbital was given in combination, the absorption of INAH at 30 minutes after the administration was noted to remain at the same degree as that with INAH alone, but it was found promoted as the time elapsed, and the rabbits became lethargic and died eventually at 3 hours and 6 hours. When the dose was increased to 0.5 g per kg, evidently promoted absorption was observed at 30 minutes after the administration, but the animals died with intoxication symptoms some time around at 45 minutes after the administration. When combined with urethane, the absorption of INAH was found slightly promoted, and the blood plasma INAH level slightly rose and was maintained relatively for a longer time. Contrary to this, no particular influence was noted in the case of the combination with magnesium sulfate.

In the cases of the enema combined with cattle bile, the blood plasma INAH level at 30 minutes after the administration was found to be the highest, and the absorption of INAH in large intestine was found to be slightly promoted, but with taurin, glycocholl or with histidine, the highest level was recorded at 1 hour after the administration, which gradually diminished showing little influence on the absorption of INAH.

In the cases of the combination with urea, thiourea or with glucose, no particular influence on the absorption of INAH in large intestine was recognized, showing the highest level at 30 minutes after the administration with gradual diminution. Unlike the above cases, in the cases of the combination with Sprase, the absorption of INAH in large intestine was found evidently promoted showing the highest level at 30 minutes after the administration, which exceeded the blood plasma INAH level in the case with the same amount of INAH alone. Tsuchimochi (9) reported that urea strongly promotes the absorption of sulfonamide preparations in large intestine, and that when 3 g per kg of urea was given as enema in combination, the absorption of sulfonamide preparation was promoted roughly to the same degree as that by the combination with chloral hydrate accompanying no serious intoxication symptoms. However, in the case of the absorption of INAH, such a remarkable promoting action was not recognized.

When a combined enema of INAH and Emulgen 106 was given, the absorption of INAH in large intestine was found clearly promoted in every case, and the blood plasma INAH level was found to be extremely high. The highest level was attained at 30 minutes after the administration, and it maintained relatively for a long time. In the cases of the combination with Hyren, promoted absorption of INAH was recognized in all cases; particularly when the amount of Hyren combined was smaller, the blood plasma INAH level was higher compared with that with Emulgen. The highest value was reached at 30 minutes after the administration which was maintained for a long time. However, when the amount of Hyren combined was 0.5 g per kg or more, the absorption of INAH was roughly the same degree as that with Emulgen, and in this case, the rabbits died during the period at 3 hours and 6 hours after the administration. In the cases of the combination with Tween 20, a clear promotion in the absorption of INAH in large intestine was noted within the period at 30 minutes and 3 hours after the administration, showing the highest level at 30 minutes after the administration.

In short, the absorption of INAH in large intestine is remarkably promoted by the combination with chloral hydrate, phenobarbital, Sprase or with various surface active agents (Emulgen 106, Tween 20 and particularly Hyren) given as enema. Recently, Matsumoto (10) of our Department introduced 3-10% solution of Hyren, Emulgen 106, Tween 20 and Span 20 into large intestine or into small intestine of rabbits to observe the influence of these substances, and confirmed that these substances do not exercise any remarkable influence on the rabbits. Following the above experiment, he also gave enemas of histamine combined with each of these substances, and recognized that all of these substances excepting Span 20 promote the absorption of histamine. Particularly, the absorption promotion by Hyren and Emulgen 106 was reported to be striking demonstrating serious intoxication symptoms in the early stage, which resulted the deaths of all excepting 1 animal during the period at 9 minutes 30 seconds and 21 minutes 30 seconds after the administration. The increment in the amount of the substance given in combination was reported to shorten the period up to the deaths of the animals and to

demonstrate a tendency of diminution in the amount of histamine absorbed. He also reported that the absorption of histamine was remarkable in the survived animal which was given Emulgen 106 combined with histamine, and that Tween 20 also promoted the absorption of histamine though more weakly compared with Emulgen 106 and Hyren. Matsumoto also reported that the increment in the amount of Tween 20 demonstrated a tendency to diminish the absorption of histamine as were observed in the cases of Emulgen 106 and Hyren. According to Fujinuma (11), Emulgen was known to show the most remarkable promotion in the absorption of calcium chloride in large intestine among 4 kinds of surface active agents (Emulgen, Hyren, Span and Tween). Fujinuma also reported that a small amount of Emulgen promotes the absorption of magnesium sulfate, too. Further, he added that these surface active agents do not demonstrate promoting action on the absorption of calcium and magnesium uniformly.

In relation to the phenomenon that the effect of certain kind of drugs is enhanced or inhibited by various surface active agents, Nakazawa (12) and Nakano (13) conducted experiments on the absorption of digitalis preparations introduced into stomach. Yamasaki (14) and Mannami (15) conducted experiments on the anthelmintic effect of cyclohexylchlororesorcinol, and obtained the following results. They concluded that, within certain range of the concentration, the surface tension of the solution is lowered as the concentration of the surface active agents is increased, and the effect of the solution is enhanced accordingly, and that, at certain concentration, the effect attains the maximum, but the effect diminishes at the concentration exceeding this. They also reported that the effectiveness turns to inhibition when the concentration is further increased. Not only in the absorption of histamine or magnesium in large intestine reported by Matsumoto or by Fujinuma, but also in the case of the present experiments, varying degree in the promoting action to the absorption of INAH was recognized according to the kinds and concentrations of the surface active agents employed.

CONCLUSION

The absorption of INAH in large intestine is remarkably promoted by chloral hydrate, phenobarbital, Sprase or various surface active agents (Emulgen 106, Hyren or Tween 20).

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