STUDIES ON ENEMA ON PENICILLIN ABSORPTION IN LARGE INTESTINE

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INTRODUCTION

Starting from the studies made on enzymological chemistry of large intestine, in our laboratory, by Abe (1), Sekino *et al.* (2) the effects of clysterized spleen components on the agglutination and platelet count of rabbit blood were observed by Yoshimoto (3) who thereby confirmed that the extracts obtained from hog and cattle spleen, and amino acid like crystalline substance ($C_6H_{13}O_2N$) isolated from cattle spleen etc. are absorbable in large intestine.

Yoshimoto investigating, further, the mechanism of acute toxinosis which followed an enema made with calcium chloride combined with biliary acids, attributed the cause, inferring from the results obtained, to the sudden increase of the blood calcium concentration and to the absorption of a small quantity of biliary acids. Detailed scrutinization of Yoshimoto's observation made on acute toxinosis was attempted by Usui (4), Hitomoto *et al.* (5) employing various kinds of calcium preparations and bile or biliary acids. Tsunoo and Kato (6) have also reported on interesting findings obtained from the experiments made on the absorption of biliary acids in large intestine. Kondo, Mochizuki, Kawai *et al.*, in addition, have studied the effect of various substances on calcium absorption in large intestine (not published yet) and their studies on the absorption of histamin and amino acids are undergoing now.

Reported herein is a part of the results obtained from the experiments made on penicillin absorption in large intestine performed in parallel with those on histamin and amino acids.

MATERIALS AND METHODS

Animals employed in the present experiments were grown-up rabbits weighing approximately 2kg, all of which accustomed to new environment and feeding of our laboratory at least one week prior to the experiments. Bleedings were started when they were hungry being starved over-night and no feeding was made while undergoing an experiment. Every precaution was taken to maintain the same experimental conditions as can be expected throughout the whole experiments.

For the determination of penicillin blood concentrations, so-called superimposed method (7, 8) (a kind of one dimensional diffusion method) was used since it is particularly convenient for the purpose of determining low concentrations espe-

cially when following their changes by hour. As the medium to be used for this purpose, defibrinated blood saturated with oxygen and the bacterial suspension were added with stir into previously dissolved agar medium in a thermostat adjusted at a temperature 45-48°C following Torii's prescription (Agar medium 20 cc, defibrinated goat blood 2 cc, culture-solution of *Streptococcus haemolyticus* (Cook-A) grown in 5% bouillon for 24 hours). The mixture was dispensed into small sterile test tubes (4.5×80 mm) by using capillary pipettes each in an amount of 0.7-0.9 cc, taking care not to cause any bubbling. When became sufficiently solid, standard penicillin (1667 u/mg) dissolved in physiological saline or a test material (blood) was superimposed in an amount of 0.2-0.3 cc.

Blood as a test material was obtained by heart puncture, the punctures being made on 6 occasions of 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours and 5 hours after clyster or injection. Bleedings were made each in an amount of 2 cc and poured into sterile test tubes. After letting the blood to coagulate in a slant position, the tubes were made to stand upright. When the serum was fully separated, the supernatant was used for the experiment. Superimposing the serum thus obtained on the medium described above, the test tubes were left to stand 24 hours in a cold chamber in order to inhibit, on one hand, proliferation of the microorganisms and, on the other, to give ample time for penicillin to diffuse. Then, the tubes were placed in a thermostat for a period of 16 hours. Potency counts were made by measuring the range inhibited of hemolysis with an accuracy of 0.5 mm.

Application was mainly made by clyster, the rabbits being fixed in a dorsal position. With an enema syringe of a capacity 20-30 cc, materials to be examined were clysterized slowly through anus and flow-out of the clysterized material was avoided as much as practicable by closing the anus.

Various substances used in combination with penicillin (Penicillin G Crystalline (Taito)) were as follows: Dextrose (Takeda Ph.), Glycocoll (Takeda Ch.), Chloral hydrate (Daiichi Ph.), Taurin (Takeda Ch.), Magnesium sulphate (Takeda Ch.), Urethane (Takeda Ch.) and Sprase (Mochida Ph.).

RESULTS

I. *Penicillin alone*

1. *Clyster with penicillin alone* (Table 1)

Rabbits weighing approximately 2 kg when clysterized with 50,000 units (10 cc) of penicillin G sodium, penicillin u/cc curves of their sera were highest at 30 minutes after the application and thereby decreased gradually showing only but a trace by the end of 5 hours.

2. *Subcutaneous injection with penicillin alone* (Table 2)

An equal amount of penicillin (50,000 units) when injected subcutaneously, showed the blood concentration curve with the highest peak at 30 minutes after the injection and thereafter diminishing gradually retained only but a trace by the end

of 4-5 hours.

II. Penicillin clysterized in combination with dextrose, glyccoll and chloral hydrate
(Table 3)

1. Penicillin in combination with dextrose and glyccoll

The amount applied were either 10 cc or 20 cc, concentrations of dextrose and glyccoll being 20% and 10% respectively. The blood concentration was highest at 30 minutes after the administration and the duration of penicillin present in the blood was 2 hours after the application while reduced to only but a trace by 3-4 hours.

2. Penicillin clysterized in combination with dextrose, glyccoll and chloral hydrate

When chloral hydrate was added to the above combination, the duration of penicillin present in the blood was prolonged additional one hour. Generally the blood concentrations were higher in comparison with those of the preceding experiments.

TABLE I

No.	Weight (kg)	Administered dosage Oxford units	Penicillin blood levels (u/cc)					
			30 ^m	1 ^h	2 ^h	3 ^h	4 ^h	5 ^h
No. 1		Penicillin G 50,000 (10cc)						
♂	2.500		0.109	0.050	0.050	0.030	0.030	Trace
No. 2		"						
♀	2.600		0.460	0.200	0.109	0.050	Trace	Trace
No. 3		"						
♂	2.500		0.580	0.260	0.200	0.130	0.050	Trace
No. 4		"						
♀	2.600		0.140	0.130	0.050	0.001	Trace	0.000
No. 5		"						
♂	2.000		0.650	0.260	0.109	0.020	Trace	Trace

TABLE II

No. Sex	Weight (kg)	Administrated dosage Oxford units	Penicillin blood levels (u/cc)					
			30 ^m	1 ^h	2 ^h	3 ^h	4 ^h	5 ^h
No. 51 ♂	2500	Penicillin G 50,000 (10 cc)	0.478	0.309	0.128	0.065	0.005	Trace
No. 52 ♂	2000	"	0.380	0.199	0.027	0.006	Trace	Trace
No. 53 ♂	2500	"	0.812	0.478	0.199	0.128	0.042	0.013
No. 54 ♀	2600	"	0.741	0.478	0.022	0.011	0.007	0.003
No. 55 ♂	2500	"	0.741	0.473	0.035	0.007	Trace	0.000

TABLE III

No. Sex	Weight (kg)	Administrated substances and dosage	Penicillin blood levels (u/cc)					
			30 ^m	1 ^h	2 ^h	3 ^h	4 ^h	5 ^h
No. 6 ♂	2300	Penicillin Dextrose 4.0 Glycocoll 2.0 (20cc)	3.091	0.011	0.003	Trace	0.000	0.000
No. 7 ♂	2300	"	1.420	0.003	Trace	0.000	0.000	0.000
No. 8 ♀	2100	" (10cc)	0.065	0.042	0.004	Trace	Trace	0.000
No. 10 ♂	2200	"	0.195	0.051	0.000	0.000	0.000	0.000
No. 11 ♂	2300	Penicillin Dextrose Glycocoll Chl. hydr. 0.3	0.741	0.330	0.128	0.001	0.000	0.000
No. 12 ♂	2300	"	0.123	0.033	0.003	Trace	0.000	0.000
No. 15 ♀	2400	Penicillin Dextrose Glycocoll (Isotonic solution)	0.389	0.250	0.169	0.087	0.066	Trace
No. 16 ♂	2150	"	0.036	0.100	0.036	Trace	0.000	0.000
No. 17 ♂	2200	Penicillin Dextrose Glycocoll (Isotonic solution) Chl. hydr. 0.3	0.195	0.253	0.112	0.000	0.000	0.000
No. 18 ♀	2700	"	0.112	0.037	0.039	0.000	0.000	0.000

3. *Penicillin clysterized in combination with dextrose, glyccoll (isotonic solution) and chloral hydrate*

Duration of penicillin present in the blood was for 2–3 hours after the administration and by the end of 3–4 hours it became a trace or disappeared.

III. *Penicillin clysterized in combination with dextrose and taurin* (Table 4)

1. *Penicillin applied in combination with dextrose and taurin (hypertonic solution)*

Proportions of dextrose and taurin per 10 cc of the mixture were 20% and 10% respectively. Duration of penicillin present in the blood was for 1 hour and by the end of 2 hours, penicillin in the blood disappeared.

2. *Penicillin applied in combination with dextrose and taurin (isotonic solution)*

The blood concentration was highest 2–3 hours after the application and even by the end of 5 hours it did not disappear.

3. *Penicillin applied in combination with taurin (isotonic solution) and chloral hydrate*

The blood concentration was highest 2–3 hours after the application and continued as long as 4–5 hours.

IV. *Penicillin clysterized in combination with morphine hydrochloride* (Table 5)

1. *Penicillin, morphine hydrochloride combination*

When applied in combination with 5 mg morphine per kg body weight, the blood concentration was highest at 30 minutes after the application and continued for a period of 2 hours.

TABLE IV

No.	Weight Sex (kg)	Administered substances and dosage	Penicillin blood levels (u/cc)					
			30 ^m	1 ^h	2 ^h	3 ^h	4 ^h	5 ^h
No. 23	♂ 2.150	Penicillin Dextrose 1.0 Taurin 0.5 (10cc)	0.427	0.125	0.000	0.000	0.000	0.000
No. 24		"	0.085	0.095	0.000	0.000	0.000	0.000
No. 25	♂ 2.080	Penicillin Dextrose Taurin (Isotonic solution)	0.033	0.033	0.109	0.095	0.075	0.056
No. 26	♀ 2.100	"	0.043	0.075	0.037	Died		
No. 27	♀ 2.620	Penicillin Dextrose Taurin (Isotonic solution) Chl. hydr. 0.3	0.185	0.250	0.250	0.313	0.257	0.213
No. 28	♀ 2.400	"	0.033	0.141	0.141	0.109	0.043	Trace

TABLE V

No. Sex	Weight (kg)	Administrated substances and dosage	Penicillin blood levels (u/cc)					
			30 ^m	1 ^h	2 ^h	3 ^h	4 ^h	5 ^h
No. 19 ♂	2.250	Penicillin Morphine hydrochloride (per kg 5mg)	0.338	0.223	0.093	0.000	0.000	0.000
No. 20 ♀	2.850	"	0.389	0.100	0.058	0.000	0.000	0.000
No. 21 ♂	1.700	Penicillin Dextrose Glycocoll (Isotonic solution) Morphine hydrochl. (per kg 5mg)	0.063	0.093	0.093	0.053	0.075	0.063
No. 22 ♀	1.560	"	0.232	0.075	0.003	Died		

2. *Penicillin, dextrose, glycocoll and morphine hydrochloride combination (isotonic solution)*

No remarkable peak of the blood concentration was observable but the duration was for a period of 5 hours.

V. *Penicillin clysterized in combination with magnesium sulphate or urethane* (Table 6)

1. *Penicillin, magnesium combination*

When penicillin was clysterized in combination with magnesium sulphate in an amount of 2.0 gm, the blood concentration of penicillin ensued was low diminishing to a trace or disappearing by the end of 1 hour. When penicillin applied in combination with 1.0 gm magnesium sulphate, the duration was 4–5 hours.

2. *Penicillin, urethane combination*

On either of the cases combined with urethane in an amount of 2.0 gm or 1.0 gm, the blood concentration followed was higher than those obtained in any other combination and in addition, the duration was over a period of 5 hours.

VI. *Penicillin clysterized in combination with bile* (Table 7)

1. *Penicillin, cattle-bile combination*

When penicillin was clysterized in combination with bile in a dose of 0.25 gm, the blood concentration ensued was highest at 30 minutes after the application and the period of duration was 2–3 hours. In case the amount of bile applied was 0.5 gm, the findings were essentially the same to the above but the blood concentration was higher. When the amount of bile was 1.0 gm, the findings were practically same to those of the case with 0.5 gm.

2. *Penicillin, hog-bile combination*

Likewise, the doses of bile employed were 0.25, 0.5 and 1.0 gm. Generally, the blood concentrations observed were slightly higher than those observed in the cases with cattle-bile and the duration was 3—4 hours.

TABLE VI

No. Sex	Weight (kg)	Administered substances and dosage	Penicillin blood levels (u/cc)					
			30 ^m	1 ^h	2 ^h	3 ^h	4 ^h	5 ^h
No. 41 ♀	2.300	Penicillin Magnesium sulphate 2.0 (10cc)	0.002	Trace	0.000	0.000	0.000	0.000
No. 42 ♂	2.330	"	0.006	0.006	0.000	0.000	0.000	0.000
No. 43 ♂	2.250	Penicillin Magnesium sulphate 1.0	0.313	0.333	0.313	0.230	0.230	0.125
No. 44 ♀	2.650	"	0.148	0.148	0.050	0.020	0.020	Trace
No. 45 ♀	2.480	Penicillin Urethane 2.0	6.761	4.168	2.238	1.445	1.258	1.258
No. 46 ♂	2.050	"	2.951	1.698	0.758	0.666	0.501	0.666
No. 47 ♂	2.150	Penicillin Urethane 1.0	4.168	2.000	1.737	0.450	0.109	0.015
No. 48 ♂	2.000	"	1.548	0.758	0.501	0.380	0.200	0.125

TABLE VII

No. Sex	Weight (kg)	Administrated substances and dosage	Penicillin blood levels (u/cc)					
			30 ^m	1 ^h	2 ^h	3 ^h	4 ^h	5 ^h
No. 29 ♂	2.450	Penicillin Cattle-bile 0.25	0.519	0.250	0.085	0.049	0.043	0.000
No. 30 ♂	2.100	"	0.183	0.075	Trace	0.000	0.000	0.000
No. 35 ♂	2.250	Penicillin Cattle-bile 0.50	0.750	0.195	0.042	Trace	0.000	0.000
No. 36 ♀	2.750	"	0.750	0.050	0.008	Trace	0.000	0.000
No. 39 ♂	2.230	Penicillin Cattle-bile 1.00	2.345	0.195	0.015	0.008	0.000	0.000
No. 40 ♀	2.350	"	1.480	0.154	0.010	0.000	0.000	0.000
No. 31 ♀	1.890	Penicillin Hog-bile 0.25	0.141	0.085	0.075	Trace	0.000	0.000
No. 32 ♂	2.090	"	0.282	0.109	Died			
No. 33 ♂	2.000	Penicillin Hog-bile 0.50	2.834	0.602	0.078	0.013	Trace	Trace
No. 34 ♀	2.570	"	1.480	0.313	0.021	0.010	Trace	Trace
No. 37 ♂	1.850	Penicillin Hog-bile 1.00	7.080	3.631	0.602	1.870	0.021	Trace
No. 38 ♂	2.000	"	2.345	0.330	0.008	0.000	0.000	0.000

TABLE VIII

No. Sex	Weight (kg)	Administrated substances and dosage	Penicillin blood levels (u/cc)					
			30 ^m	1 ^h	2 ^h	3 ^h	4 ^h	5 ^h
No. 49 ♀	2.400	Penicillin Sprase 5000 V.U.M.	0.501	0.204	0.030	0.050	Trace	0.000
No. 50 ♂	2.350	"	0.645	0.257	0.204	0.030	0.011	0.030

VIII. *Penicillin clysterized in combination with sprase* (Table 8)

The blood concentration was highest at 30 minutes after the application and by the end of 4-5 hours, it diminished to a trace or disappeared.

SUMMARY

Regarding rectal application of penicillin, Loewe *et al.* (9) reported, in 1945, that they proved the presence of penicillin in the blood of 12 cases out of 14 applied with 300,000-1,000,000 units. In our experiments made by applying penicillin alone through subcutaneous route or rectum, it was found that the blood concentration was the highest at 30 minutes after the application thereafter diminishing gradually and showing only but a trace or disappearing by the end of 4-5 hours. When penicillin was clysterized in combination with dextrose and glyccoll, the blood concentration was likewise highest at 30 minutes after the application but reduced to a trace or disappeared by the end of 2-3 hours, while when isotonic, the duration of the period penicillin present in the blood was prolonged as long as 4-5 hours, whereas, when chloral hydrate was combined in addition to the above, the penicillin disappeared from the blood stream by the end of 3-4 hours. In case penicillin was clysterized in combination with dextrose and taurin, and when the mixture was hypertonic, penicillin in the blood disappeared within around 2 hours, but when isotonic, the period of penicillin present in the blood became more than 5 hours. Addition of chloral hydrate, further, did not show any appreciable difference. In case penicillin was clysterized in combination with dextrose, glyccoll and added with morphine, when isotonic, the period of penicillin present in the blood stream was approximately 5 hours, but when morphine was added alone, the period was not more than 3 hours. Magnesium sulphate did not show any particular effect. While on the other hand, penicillin clysterized in combination with urethane showed the highest blood concentration at 30 minutes after the application and the penicillin was confirmed present over a period of 5 hours in the blood stream with a remarkably high concentration.

In case penicillin was combined with bile (cattle and hog), though the blood concentration was high, the duration of penicillin proved present in the blood was relatively short. When penicillin was clysterized in combination with sprase (diffusion factor), the blood concentration was highest at 30 minutes after the application and disappeared by the end of 4-5 hours.

The absorption of calcium chloride in large intestine was largely promoted when combined with bile, dextrose, amino acids, chloral hydrate etc. (Yoshimoto, Usui, Hitomoto, Kato, Kondo *et al.*), but was not promoted in the combination with urethane, whereas, in the case of penicillin, the absorption was remarkably promoted when clysterized in combination with urethane showing high and prolonged blood concentration.

Further investigations are undergoing in our laboratory in order to find out

a still more adequate substance for promoting the absorption of penicillin in large intestine.

CONCLUSION

Upon clysterizing penicillin in combination with various substances (dextrose, glycocoll, chloral hydrate, taurin, morphine, magnesium sulphate, urethane and sprase), urethane was found to promote extremely the absorption of penicillin showing high blood concentration of longer duration in comparison with other substances.

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