# THE BILIARY EXCRETION OF ANTIBIOTICS AND SULFONAMIDES AND THE EFFECT OF CALCIUM PARA-OXYBENZOATE THEREUPON

MINORU MIZUTA

*First Medical Clinic of the Yamaguchi Medical School, Ube*
*Director: Prof. Nobuo Mizuta*

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Several dye-stuffs, such as merucurochrome 222 soluble, trypaflavin, flavicid, etc., have hitherto been studied for the chemotherapy of infections of the bile ducts, but inasmuch as disinfecting activities are remarkably weakened by passing through the liver (Hill and Skot[1]; Takahashi[2]), a drug that maintains a high concentration in the bile has been required.

Although the recent development of sulfonamides, penicillin and streptmycin seems to meet our expectation, these have been known to be excreted chiefly into the urine, and the amount of excretion through the bile is reportedly very small (Fukushima and Mototsu[3]). Therefore, it may be purposeful if we could increase the concentration of these chemicals in the bile.

It is also a known fact that some of the aromatic fatty acids accelerate the dye-excreting functions of the liver (Inagaki[4]), and calcium para-oxybenzoate (POB) has come to be applied in medical practice (N. Mizuta[5]).

I attempted to investigate the influence of excretion of antibiotics and sulfonamides, in expectation of its favorable effect on the excretion of these antibiotics into the bile, if applied to the treatment of infected bile ducts.

## METHODS AND MATERIALS

(1) Adult rabbits were nephrectomized bilaterally and glass canulae were inserted into their common bile ducts. 0.9 per cent saline or 3 per cent aqueous solution of POB were injected, both in a dose of 2.0 ml per kg body weight*. Thirty minutes thereafter either one of the antibiotics—penicillin (potassium or sodium salt), dehydrostreptomycin or sulfonamides** were administered through their ear-vein, and the bile was fractionatedly accumulated at one hour intervals for two hours (in case of

* It was verified by control experiment, that 2.0 ml per kg body weight of 3% POB was adequate for rabbits. Namely, in the experiment with POB the rate of dye excreted during two hours was 46.6% and the time of its initial appearance in the bile was, in the average of the eight bilaterally nephrectomized rabbits, 1'39", while in the control 20.8% and 2'39" respectively, in 4 cases. The dye-stuff and the dose employed was 1.0 ml per kg body weight of 0.6% aqueous solution of phenolsulfonphthalein.

** 50 mg in aqueous solution, a dose as small as possible, since sulfonamide promotes liver function.[11]

penicillin and streptomycin) or at half-hour intervals for two hours (in case of sulfonamides). Urine was also collected through a Nelaton's catheter by washing the bladder with a certain amount of the physiological saline solution.

(2) The concentration of penicillin excreted into each bile fraction was estimated by the cup-method, an approved method for this purpose by the Japanese Welfare Ministry[6], and the amount of penicillin excreted into the bile and its percentage to the injected amount were determined.

(3) The concentration of penicillin in the serum was estimated by the pile-method (Kawakami[7]).

(4) The concentration of streptomycin excreted into the bile was estimated by the pile-method using Proteus vulgaris (Torii and Kawakami[8]).

(5) Sulfonamide excreted into the bile was estimated electro-photometrically by Tsuda's method[9], using $\beta$-diethyl aminoethyl-$\alpha$-naphthyl amino oxalate, after treating with Carryer-Ivy's[10] procedure. Free sulfonamide (F) and total sulfonamide (T) were determined and the percentage of conjugation, $(100\text{-}F/T \times 100)\%$, was caluculated.

(6) The concentration of sulfonamide in the blood was estimated by Tsuda's method after treating with trichloric acetic acid.

(7) Each result was indicated in figures which were the average of two or three cases.

## Results and Discussion

The results obtained are presented in Figures 1–4.

*1. Penicillin* (Figures 1 and 2)

Little has been known about the biliary excretion of penicillin but that the penicillin concentration in bile is slightly higher than in blood.

(a) The biliary excretion of penicillin and the influence of POB upon it in the bilaterally nephrectomized rabbits: As will be seen from Fig. 1, the biliary concentration of penicillin, though distinctly lower than its urinary concentration, ascends with the increase in the amount of penicillin administered, while its excretion rate remains relatively constant. The excretion of penicillin-K is enhanced by the injection of POB and in some instances it increases about 40 per cent, particularly in the first one hour, thus determining the total amount of excretion. The excretion of penicillin-Na is also accelerated by POB, though somewhat less conspicuously than is penicillin-K.

(b) The biliary excretion of penicillin and the influence of POB upon it in the non-nephrectomized rabbits: Fig. 1 indicates that the penicillin excretion rate in these animals amounts to 1.19 per cent for three hours, the major part being excreted in the first hour. This is augmented to 2.52 per cent by POB injection.

The urinary excretion of penicillin reaches in amount nearly 30 to 60 times as much as its biliary excretion, and its major excretion occurs also during the first hour.

Contrary to the biliary excretion, any significant increase by POB is not noticed.

(c) The influence of POB upon the penicillin concentration in blood: It has been said by some authors that benzoic acid and sodium benzoate contribute to the retention of penicillin in the blood. Inasmuch as POB resembles these in chemical structure, an investigation was attempted to elucidate whether POB has the similar effect. This was confirmed by the results illustrated in Fig. 2, in which POB secures the blood penicillin concentration at higher level when it is administered following the second penicillin injection.

Fig. 1 Biliary Excretion of Penicillin.

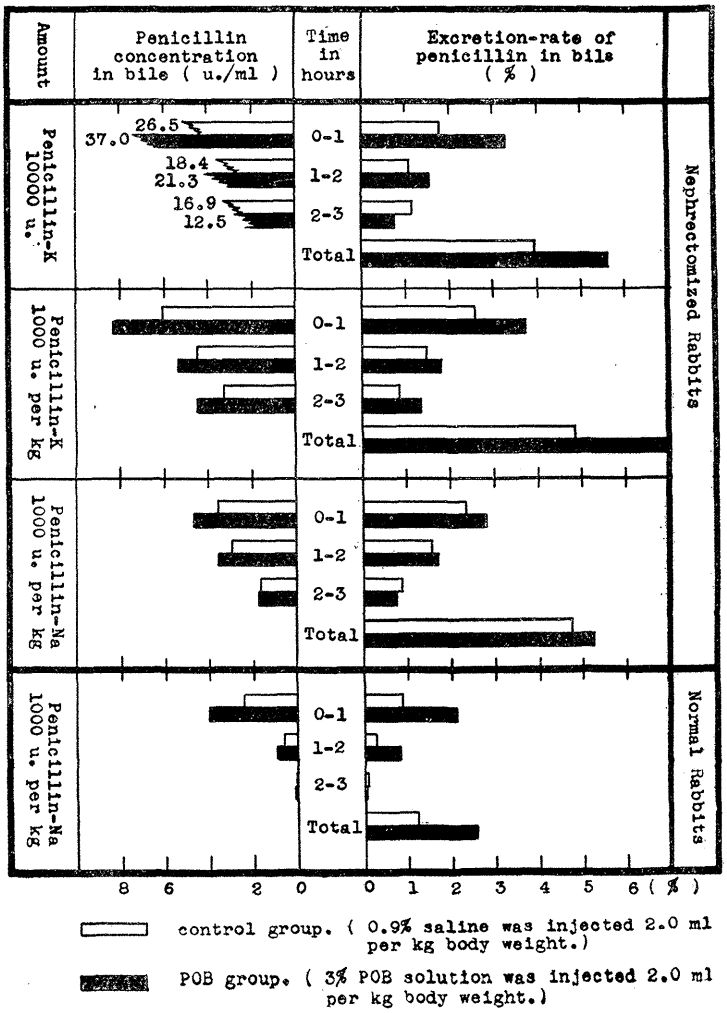
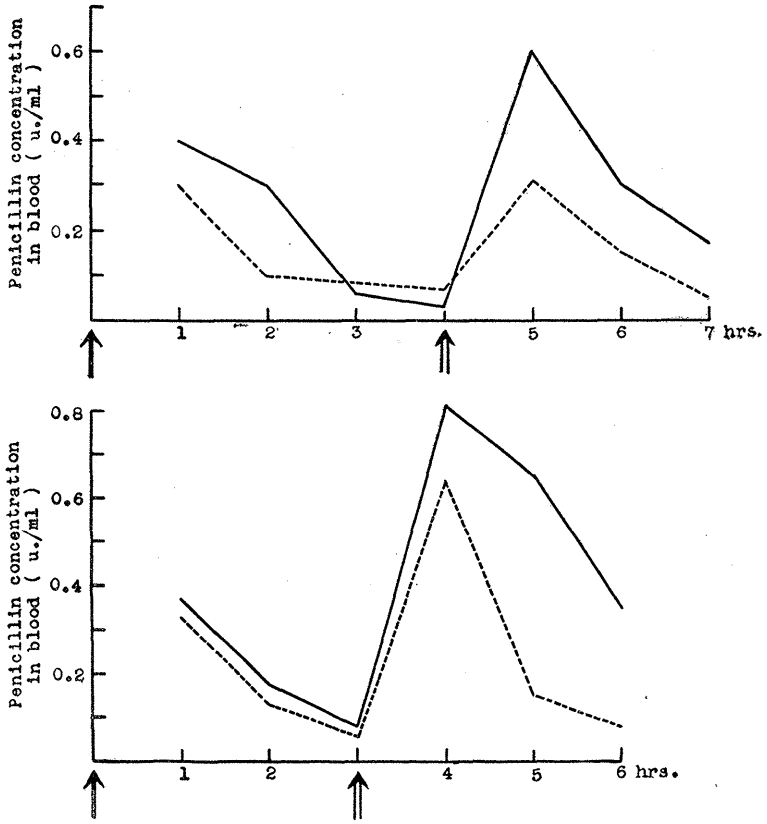


Fig. 2 Influence of POB upon the Penicillin Concentration in Blood.




Solid line indicates a control case, which recieved intramuscular injection of penicillin-Na (2500 u.) at the point (↑), and recieved the second injection of penicillin-Na (2500 u.) and 0.9% saline 2.0 ml per kg body weight at the point (⇑).
Broken line indicates a case, which recieved intramuscular injection of penicillin-Na (2500 u.) at the point (↑), and recieved the second injection of penicillin-Na (2500 u.) and 3% POB 2.0 ml per kg body weight at the point (⇑).

*2. Streptomycin* (Figure 3)

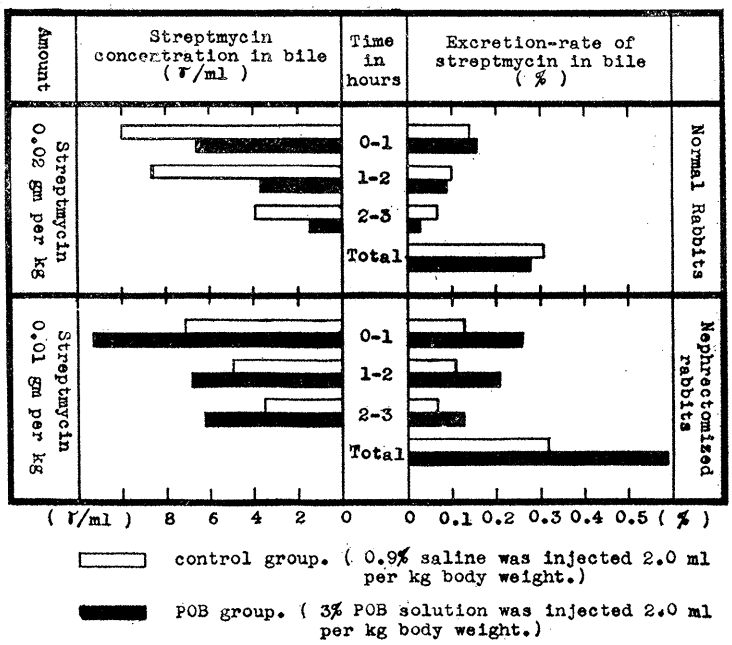
(a) The biliary excretion of streptomycin and the influence of POB upon it in the non-nephrectomized rabbits: As will be noticed from Fig. 3, the excretion rate of streptomycin into the bile during the first three hours following its administration (0.02 gm per kg body weight) barely attains 0.31 per cent of the amount injected, corresponding to only one-third of that in the case of penicillin. But special mention is made of the fact that streptomycin is excreted for a longer time in almost constant concentration than is penicillin whose maximal excretion takes place in the first one hour, and its excretion is, if anything, reduced by the POB injection.

The urinary excretion of streptomycin varied in such wide range that reliable

results were never obtained.

(b) The biliary excretion of streptomycin and the influence of POB upon it in the bilaterally nephrectomized rabbits: Fig. 3 reveals that POB injection increase the streptomycin excretion (streptomycin is administered 0.01 gm per kg body weight) from the level of 0.32 per cent up to 0.59 per cent, that is, to 1.8 times as high as in the control group which recieves no POB. Accordingly POB exhibits also a latent promoting action to the biliary excretion of streptomycin in nephrectomized rabbits.

Fig. 3 Biliary Excretion of Streptmycin.




*3. Sulfonamides* (Figure 4)

The concentration of sulfonamide in the bile did not greatly differ from that of the blood.

As far as the therapeutic effect of sulfonamide is concerned, free type of sulfonamide is believed to be more important than the conjugated type, and it does not seem weise to apply POB to the sulfonamide treatment of infections of the bile ducts.

POB has the ability to increase the conjugation rate of sulfonamides, except for sulfathyazole, sulfadiazine and sulfamethyldiazine. However its inhibiting effects upon these three are very small and unlikely. These irregular results can be explained by the fact that almost all sulfonamides have an ability to stimulate the liver function[11], and in the stimulated condition of the liver, further acceleration of the liver function will not be exerted by means of POB[12].

Promoting the conjugation of sulfonamide is regarded to be the increase in detox-

Fig. 4 Biliary Excretion of Sulfonamides.

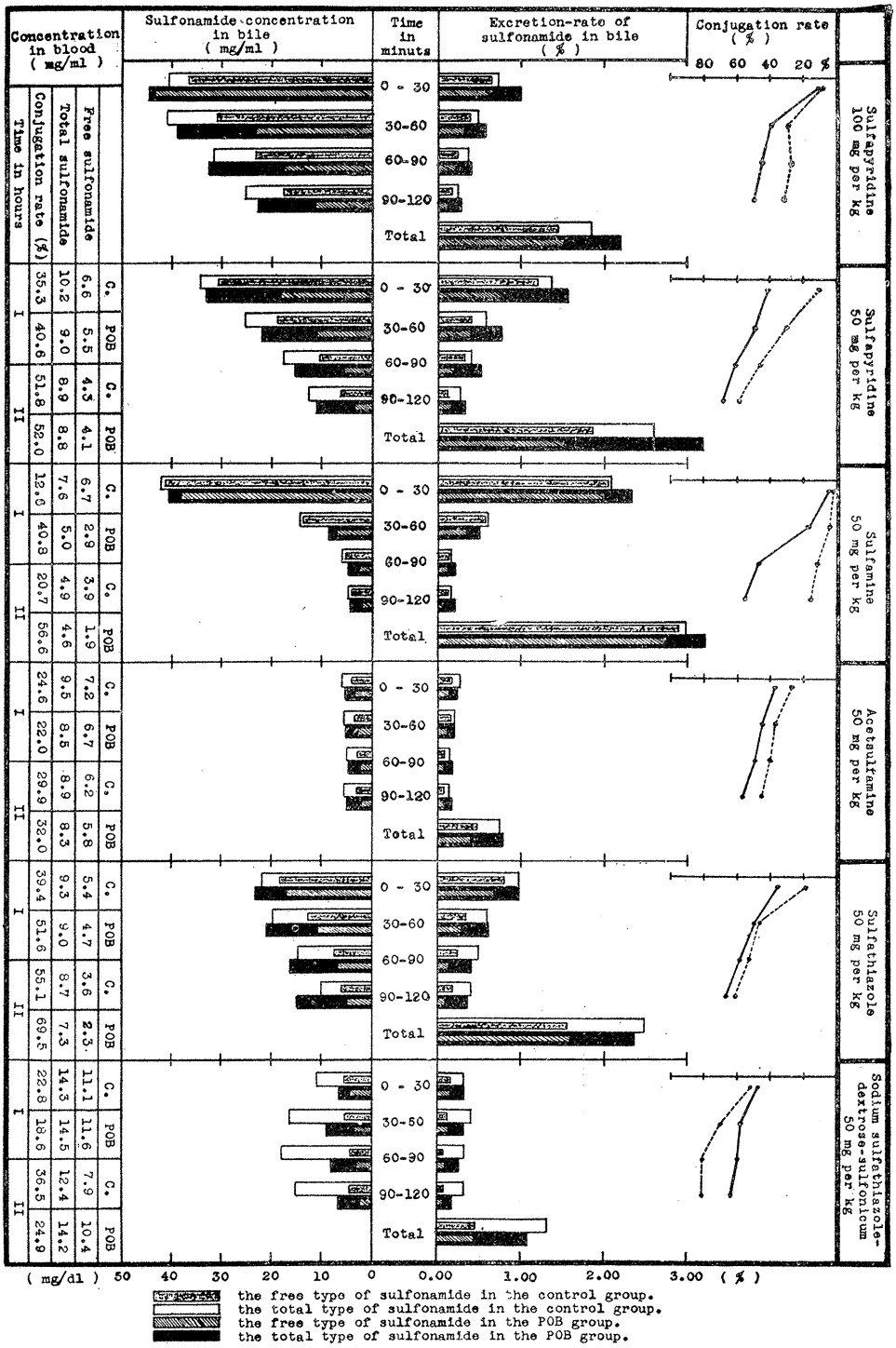
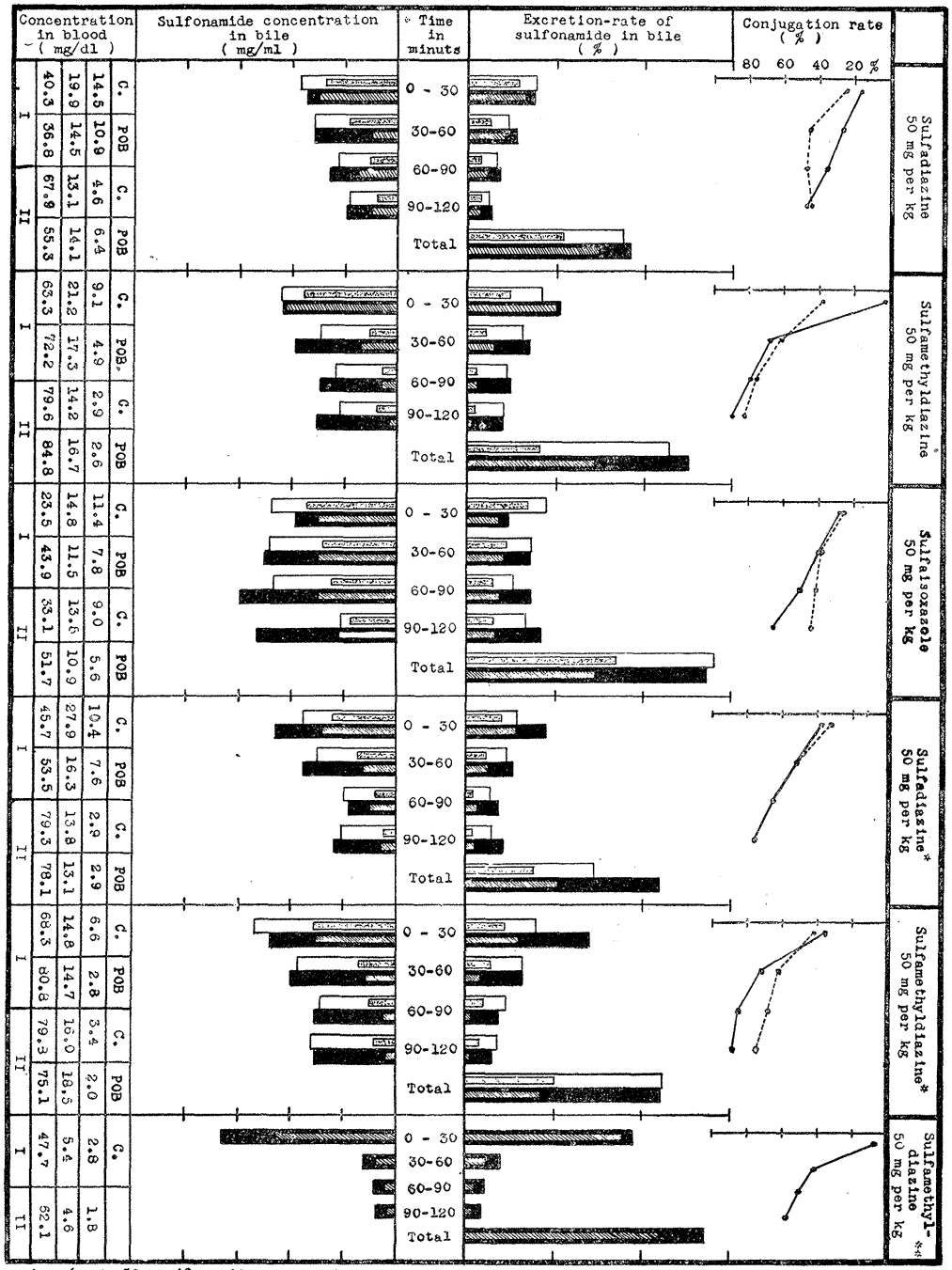


Fig. 4 Continued




| | | Sulfadiazine 50 mg per kg | | | | Sulfamethyldiazine 50 mg per kg | | | | Sulfaisoxazole 50 mg per kg | | | | Sulfadiazine* 50 mg per kg | | | | Sulfamethyldiazine* 50 mg per kg | | | | Sulfamethyl-diazine 50 mg per kg ** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C. | POB | C. | POB | C. | POB | C. | POB | C. | POB | C. | POB | C. | POB | C. | POB | C. | POB | C. | POB | C. | |
| I | 0 - 30 | 14.5 | 10.9 | 9.1 | 4.9 | 11.4 | 7.8 | 10.4 | 7.6 | 6.6 | 2.8 | | | | | | | | | | | | |
| | 30-60 | 4.6 | 6.4 | 2.9 | 2.6 | 9.0 | 5.6 | 2.9 | 2.9 | 3.4 | 2.0 | | | | | | | | | | | | |
| | Total | 40.3 | 36.8 | 63.3 | 72.2 | 23.5 | 43.9 | 45.7 | 53.5 | 68.3 | 80.3 | 47.7 | |
| II | 0 - 30 | 19.9 | 14.5 | 21.2 | 17.3 | 14.8 | 11.5 | 27.9 | 16.3 | 14.8 | 14.7 | 5.4 | |
| | 30-60 | 13.1 | 14.1 | 14.2 | 16.7 | 13.6 | 10.9 | 13.8 | 13.1 | 16.0 | 18.5 | 4.6 | |
| | Total | 67.9 | 55.3 | 79.6 | 84.8 | 33.1 | 51.7 | 79.3 | 78.1 | 79.3 | 75.1 | 52.1 | |

Solid line indicates the conjugation rate in the POB group.
Broken line indicates the conjugation rate in the control group.
* Rabbits recieved 2.0 ml per kg body weight of 40% glucose solution and 3% POB solution instead of POB only.
** Rabbits recieved 2.0 ml per kg body weight of 10% solution of natrium dehydrocholicum instead of POB.

Conjugation rate = ( 100 - F/T )×100%  C. : Control

ifying activity, therefore, a larger dose of sulfonamide may not be permitted by combining POB with this drug.

The amount of free type of sulfonamide excreted into the bile during 2 hours, stands in the following condescending order, from the hight to the lowest (Fig. 4): sulfamine → sulfapyridine → sulfaisoxazole → sulfathiazole → sulfadiazine → sulfamethyldiazine → acetsulfamine → sodium sulfathiazole-dextrose-sulfonicum.

From the view point of obtaing the excretion of the free type of sulfonamide for a long period without losing its concentration in the bile, sulfaisoxazole, sulfapyridine and sulfathyazole are best, in that order. Sulfamine was excreted very slightly from the second hour, though very remarkablly in the first hour. It is reasonable to avoid the use of sulfapyridine for the infections of bile ducts, which is usually accompanied with the injury of liver function, as sulfapyridine damages the liver by consecutive use[8].

From the facts mentioned above and the fact that sulfathyazole and sulfaisoxazole are not so harmful to the liver[8], these two sulfonamides are regarded to be purposeful for the treatment of infections of bile ducts. Furthermore, inasmuch as sulfaisoxazole has a strong antibacterial activity on various kinds of bacilli, especially to coli bacilli, the use of sulfaisoxazole is best suited for this purpose.

The use of natrium dehydrocholicum instead of POB, the former accelerating the excretion of bile, induces the excretion of the less concentrated bile and increases the conjugation rate, but such increase in sulfamethyldiazine was not obtained. Therefore, it is of no use to apply natrium dehydrocholicum, combined with sulfonamide, for disinfection of bile ducts, except for the washing of bile ducts.

With regard to the detoxification of sulfonamide in the body, it has been said that glucuronic conjugation and acetiration are the possible ways of detoxification. The application of both glucose only and the combination of glucose and POB accelerated the conjugation rate of sulfadiazine and sulfamethyldiazine, whereas POB only gave opposite results. This may be explained by the glucuronic conjugation, and it is purposeful to apply POB combined with glucose to the treatment of sulfonamide poisoning.

## Conclusion

1) The excretion of penicillin, streptomycin and sulfonamide in the bile was studied in rabbits.

2) The excretion of penicillin and streptomycin into the bile, especially of the former, was promoted by the injection of calcium para-oxybenzoate (POB).

3) Sulfaisoxazole is most purposeful among sulfonamides in the treatment of infections of the bile ducts.

4) POB did not increase the excretion of sulfonamide into the bile, but accelerated the conjugation of it.

5) It is proposed that a larger amount of sulfonamide may be used with the

help of the combination of POB and glucose.

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## REFERENCES

1) HILL AND SKOT: *Arch. Intern. Med.* **35**: 503, 1925.
2) TAKAHASHI: *Japanese Jour. Gastroenterology,* **1**: 169, 1929.
3) FUKUSHIMA AND MOTOTSU: *Rinsho naika shonika* (in Japanese), **4**: 534, 1949.
4) INAGAKI: *Japanese Jour. Gastroenterology,* **9**: 108, 122, 136, 1937,
5) N. MIZUTA, O. MATSUO AND M. MIZUTA: *Bull. Yamaguchi Med. School,* **1**: 12, 1953.
6) *Penicillin* (in Japanese), **2**: 154, 1949.
7) KAWAKAMI: *Penicillin* (in Japanese), **1**: 445, 1949.
8) TORII AND KAWAKAMI: *Penicillin* (in Japanese), **2**: 719, 1949.
9) TSUDA AND MATSUNAGA: *Jour. Pharmacology* (in Japanese), **62**: 362, 1942.
10) CARRYER AND IVY: *Jour. Pharm. & Exp. Therapy,* **166**: 302, 1939.
11) M. MIZUTA: *Bull. Yamaguchi Med. School,* **1**: 31, 1953.
12) M. MIZUTA: *Bull. Yamaguchi Med. School,* **2**: 116, 1953.