Fundamental Studies on the Metabolism of Rifampicin (RFP)

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Introduction of Rifampicin (RFP) into leprosy field has brought about a marked improvement to the clinical treatment of leprosy. This drug as a simple antimycobacterial substance can be distinguished from DDS which possesses several anti-inflammatory actions as already published previously¹⁾. As was mentioned in the precedent general view²⁾, this drug belongs to a sort of long-acting antibiotics through its entero-hepatic circulation as in the case of Novobiocin. Hereon, the long-term preservation of whole body distribution expressed generally by action area is an important factor for the systemic infectious diseases such as leprosy.

The inactivation of a drug under the *in vivo* metabolism can be classified into two, that is, the conjugation and the change in the skeleton causal to its efficacy. In the former case the efficacy of the drug can be recovered by the reversible demasking while it is real inactivation in the latter. In the case of RFP, the *in vitro* effects of its main metabolites such as desacetyl RFP (DARFP) and the quinone form (RFPQ) are known to be nearly comparable to that of RFP itself. In order to presume the *in vivo* preservation of RFP activity, the metabolism of each RFP metabolite was individually examined by the use of tracer method.

Materials and Methods

1. Synthesis of standard materials. Half a mCi of ¹⁴C-RFP (¹⁴C-CH=aminomethylpiperazinyl, 2.7mCi/mM. 3.28 μ Ci/mg) was kindly supplied by Daiichi Pharm. Co.

¹⁴C-DARFP and ¹⁴C-RFPQ; According to the authentic method summarized in the precedent general view,¹⁾ these were synthesized and purified on buffered silica plate of 2 mm thickness. Each standard material showed single sharp peak radiochemically, pigmentally, and by UV-irradiation after developed on TLC plate with CHCl₃-MeOH (9:1) or Me₂CO. Sp. Act., DARFP, 2.2 mCi/mM; RFPQ, 1.4 mCi/mM.

¹⁴C-formyl RFP (RM-AF): When RFP is hydrolyzed under acidic side by the authentic method (in 0.1 N HCl-0.1 M Na-citrate, pH 2.2 at 90°C for 4-6 hrs.), numerous pigmental belts are seen on TLC plate bufferered at pH 6.0. Since the adsorption of RM-AF on the silica powder is so tight that it is difficult to extract RM-AF with CHCl₃. Therefore, the crude reaction mixture after hydrolysis was extracted with CHCl₃ and it was once adsorbed on silica powder. The powder was repeatedly washed with CHCl₃ to eliminate unchanged

RFP and the other side-products. Then, powder was extracted with EtOH and evaporated rapidly below 40°C because CHO radical of RM-AF changes into its diethylacetal within 3 hrs. Thus, RM-AF of single peak could be isolated.

RFP-4-O-adamantane carboxylate (ADP): Caused by the following reasons this substance was synthesized. a) to avoid the rapid oxidation of RFP to RFPQ, b) to increase further the liposolubility of RFP, c) to presume stereochemically the position of glucuronide conjugation.³⁾ 1-adamantyl chloride (AdCl, 50 mg, ca 250 μ M, Tokyo Kasei Co.) was dissolved in 1 ml of ice-cold pyridine. To this, a solution of ¹⁴C-RFP (42 mg, ca 50 μ M, 135 μ Ci dissolved in 3 ml of pyridine-benzene (1:1) including 10 mg of ascorbic acid and one drop of 1% N-methyN'-aminopiperazine) was dropwisely added and kept for 2.5 hrs. at 5°C. It was diluted with 10 ml of CHCl₃ and repeatedly washed with 0.1 M phosphate, pH 7.0. The solvent was evaporated below 40°C and the residue was dissolved into small volume of CHCl₃ and developed on a silica plate (Kieselgel, thickness 2 mm, buffered with phosphate, pH 6.5) with CHCl₃-benzene (5:3).

Five pigmental belts were separately detected, Rf 0.92 yellow and slight, Rf 0.79 orange and slight, Rf 0.58 orange and very thick, Rf 0.33 violet and slight, and Rf 0.25 reddish and slight. The third main pigmental belt was scraped off and extracted with CHCl₃. After evaporation of CHCl₃ *in vacuo*, it remained yellow orange powder. It showed Rf value of 0.76 when developed with n-PrOH-CHCl₃-Dioxane (1:3:1) saturated with 0.1 M phosphate, pH 6.5. While Rf values of the other standard materials were as follows: RFPQ, 0.66; DARFP-quinone (DARFPQ, synthesized from DARFP chromatographically), 0.54; RFP, 0.41; DARFP, 0.26. Sp. Act., 0.96 mCi/mM. Similarly, by the use of non-labeled RFP, non-labeled standard of ADP was synthesized. Since the reaction scale was larger than that of labeled case, the purification on TLC plate was repeated two times. Yellow orange needles, mp 171-174°C (decomp.). *Anal.* Calcd. for C₅₄H₇₂N₅O₁₃: C%, 65.83; H%, 7.36; N%, 5.68. Found; C%, 66.20; H% 7.70; N%, 5.02.

Except the lack of 4-OH peak, the NMR, spectrum of ADP was resemble to that of RFP as shown in Fig. 1.

2. Whole body autoradiography(WBARG). Five μ Ci of each standard material suspended in Tween 80-CMCNa/5% EtOH was dosed orally to mice by catheter and the mice were timewisely sacrificed by freezing after ether anesthesia. Each whole body slice of 40 μ thickness by Auto-Cryotome NA 200F was dried and contacted with Fuji IX covering a period of 6 weeks. On the other side, 2 μ Ci of RFP was intramuscularly injected to Wistar rat in order to analyze the urinary RFP-glucuronide (RFPQ). After the collection of urine for 4 hrs., the rat was sacrificed and the slices were contacted for 4 weeks.

3. Radiochemical analysis. The biological materials to be analyzed are picked out from residual frozen blocks and homogenized with 5-20 fold volumes of 60% MeOH/0.05 M phosphate buffer, pH 7.0 and centrifuged at 6900 $\times$ G for 15 mins. A portion of the supernatant was mixed with Bray scintillator and the radioactivity was measured by Aloka LSC 502 scintillation counter. In the cases of liver, kidney, gut materials, and muscle, the residual supernatant was evaporated to dryness below 40°C, re-extracted with a small volume of

Fig. 1. NMR Spectrum of ADP Compared with That of RFP

Me_2CO , and centrifuged. A portion of the supernatant was developed on TLC and the metabolites were examined by scraping off the silica powder on TLC plate.

The adsorption of RFP series on calf serum was examined as follows: 2.5 μCi of each ^{14}C -labeled standard material/ EtOH was nearly dried *in vacuo* and it was mixed with 2.5 ml of calf serum. After the incubation at 37°C for 30 mins., it was developed through Sephadex G 200 (particle, size, medium; column size, 3×30 cm) with 0.001 M Na-K phosphate buffer, pH 7.55 containing 0.9% NaCl. Except the case of ^{14}C -RFPQ, 0.05% ascorbic acid was further added to the developing buffer. A portion (0.2 ml) of each effluent (5 ml) was used for measuring the radioactivity by liquid scintillation counter.

4. **Color densitometry.** Ozumor 82 densitometer was used.

Results and Discussion

1. **Presumable position of conjugation.** Coincident with the lack of 4-OH peak of ADP by NMR, ADP was far stable than RFP and the oxidation to its quinone form could not be detected by the usual quinone formation procedure. Thus, masking at 4-OH of RFP by Ad radical was apparent. Besides the synthesis of ADP, the reaction of DARFP and Ad or smaller radicals such as nicotinyI was examined. Different from the case of ADP, the reaction of AdCl and DARFP produced two main pigmental belts by both of developments through Sephadex LH-20 with CHCl_3 - EtOH (2:1) and on Kieselgel TLC plate with CHCl_3 -

MeOH (9:1). The Rf values in the latter method were 0.91 and 0.80 while DARFP was 0.69 though the complete isolation of these orange yellow products could not be achieved. The reaction of nicotiny chloride and DARFP produced six pigmental belts other than DARFP and DARFPQ, when the crude products were developed by the former method. On the other hand, the reaction of RFP and 4-acetoaminobenzene sulfonylchloride (ABSCl) produced the chromatogram quite resemble to that by the reaction of ABSCl and DARFP, suggested the compulsory introduction of ABS radical to RFP. In RFP skeleton, there is a steric hinderance among 23-OH in macrolide ring and 1-OH in naphthol ring and an electric disturbance among 8-OH in naphthol ring and 1-OH (peri-effect). Therefore, the introduction of Ad radical into the space surrounded by both of macrolide and naphthol rings may be disturbed stereochemically because Ad is known to be the representative tetrahedral structure. While, 4-OH in the naphthol ring and 25-OH (DARFP) are stereochemically indifferent from any other part of RFP structure. The production of two main pigmental belts in the case of AdCl-DARFP reaction might be caused by the lack of acetyl radical at 25-OH, suggesting the introduction of Ad to 25-OH in addition to 4-OH. In the case of smaller radicals such as nicotiny, the introduction into the space such as 21-OH may be far easier thus many products were occurred and the individual isolation of them became difficult. In the same time, the compulsory substitution of ABS radical for acetyl of 25 position was associated. Therefore, when standing on the stereochemical view, the probable first position of RFP to be easily conjugable *in vivo* is presumed to be 4-OH.

2. Whole body autoradiography. According to the mutual difference in specific activities of ^{14}C -labeled standard materials, the dosage of every standard material was mutually different such as 1.5 mg (RFP), 1.8 mg (DARFP), 2.9 mg (RFPQ), and 4.2 mg (ADP) per 5 μCi to each mouse.

RFP: The enough whole body distribution could be seen only at 1 hour after the oral dose. Already at 4 hours, the radioactivity was localized to hepatic area alone. However, even after 12 hours, this localized distribution continued. After 24 hours, the radioactivity in gut materials seemed to be higher than that in the liver. In the case of 4 hours' rat autoradiogram, it was also found to be localized to entero-hepatic area.

DARFP: Throughout the slices at 1, 4, 7.5, 12, and 24 hours, the radioactivity was found to be localized to the entero-hepatic area alone.

RFPQ: Caused by the shortage of ^{14}C -RFPQ, the number of mice used were necessarily restricted to only two. The radioactivity localized to entero-hepatic area apparently continued from 4 to 12 hours.

ADP: The process of radioautograms from 1 hour to 24 hours was resemble to that of DARFP cases.

3. Radiochemical analysis. As shown on Fig. 2., DARFP rapidly diminished in the liver. The poor distribution of ADP suggested the low absorption from the gut.

Since numerous reports were published concerning the metabolism of RFP,^{4,5)} the interesting findings alone are summarized in Fig. 3.

The total recovery rate, especially the urinary one during 24 hours is known to be gene-

Fig. 2. Prosperity and Decay of Radioactivity in the Liver Autopsied from Residual Frozen Blocks of Mice after the Dose of Respective ^{14}C -labeled Standard Materials

upper rank: the change in RFPQ/RFP rate of RFP group with the lapse of time
middle rank: the change in DARFPQ/DARFP of DARFP group
lower rank: the change in the isolation of RFP from ADP (RFP/ADP) of ADP group
Fig. 3 A Result of Radiochemical Analysis of some Biological Materials Autopsied from Residual Frozen Blocks of Mice

rally low in the case of RFP. However, throughout the radiochemical analysiss, the urinary/fecal recovery rates were presumed to be lower in RFP analogues than in RFP. This

tendency was especially marked in ADP case. From Eig. 3., it may be suspected the followings.

a) The content ratios of RFPQ and DARFPQ increased with the lapse of time, gradually in the liver and since 7.5 hours in the gut.

b) The quinone formation of DARFP group was more predominant than that in RFP group.

c) RFP can be freed from ADP *in vivo*. However, since 7.5 hours, the increase of freed RFP could not be detected apparently, suggesting also the low absorption of ADP.

4. Inhibitory effect of protein on the oxidation of RFP to RFPQ. This effect had already been suspected when RFP was incubated with a cell-free extract of NQ bacilli, in order to examine the desacetylation of RFP by bacteria. Such a desacetylation could not be detected but the marked inhibition to the oxidation was observed⁶⁾. Indeed, merely a part of RFP can be changed *in vivo* into RFPQ. It may be caused by the action of various reductases as if equilibrating to the oxidation. However, in spite of the finding that RFP is rapidly oxidized in hydrophilic media without ascorbic acid especially under electrophoresis⁶⁾, the fact that it can be preserved comparatively in calf serum was also experienced. Then the reduction of RFPQ by cysteine as a model of SH terminal residue of protein was examined. The solution of RFPQ (500 μ g/20 ml of 0.1 M phosphate, pH 7.0) was mixed with 500 μ g of cysteine/20 ml of 0.1 M phosphate, pH 6.0 and the mixture was quickly extracted with CHCl_3 after 15 seconds or 5 minutes.

After the evaporation of solvent *in vacuo*, the residue was developed on TLC and scanned colorimetrically. As shown on Fig. 4., about half of RFPQ reduced to RFP within 15 seconds can be seen. After 5 minutes, the reduction to RFP was found to be complete. On the other side, the heat sterilization completely changed RFP into RFPQ. Therefore, numerous reports concerning the antibacterial effect of RFP may inform us of RFPQ effect. Naturally, there are various substances *in vivo*, for example, such as those capable of accelerating the oxidation of RFP⁷⁾ while it is difficult to isolate the completely pure RFP even *in vitro*. But this finding may give us an explanation for only partial oxidation of RFP *in vivo*.

5. Adsorption of RFP series on calf serum. Similarly to the case of WBARG, mg% of each standard material to be adsorbed was mutually different caused by the difference of specific activities, 30 mg% (RFP), 36 mg% (DARFP), and 58 mg% (RFPQ). Since the specific activity of RM-AF could not be determined caused by the deficiency, it was adsorbed merely standing on the radioactivity alone (2.5 μ Ci). As shown on Fig. 5 (a, b), all of examined RFP series were adsorbed on the first protein fraction which contains mainly IgM. Nakagawa had reported the following four points⁸⁾; (i) RFP was presumed to be adsorbed on β -globulin. (ii) It was suggested that DARFP adsorbs additionally on α_2 -globulin. (iii) The protein component affinitive to RFPQ could not be found out. (iv) RM-AF adsorbed tightly to albumin. These findings were mainly based on paper electrophoresis where protein molecule is polarized. While in the case of molecular filtration, since the serum is diluted by a large volume of buffer, the weak bondage is freed and the tight

Fig. 4. Inhibitory Effect of Cysteine as a Model of Terminal Residue of *in vivo* Protein on the Oxidation of RFP to RFPQ and the Complete Oxidation of RFP to RFPQ by Heat Sterilization, measured Densitometrically

Fig. 5. Adsorption of RFP Series of Calf Serum Examined through Sephadex G-200 Column

adsorption alone can remain and is detected. Since the first protein fraction includes both of α - and β -globulin in a rate of $\alpha \gg \beta$ at its peak, this result is never contradictory to (i) and (ii). However, since RFPQ is liposoluble more than DARFP and even than RFP, and generally quinones are known to be the substances tightly adsorbing on protein, the result (iii) seems to be exceptional or even contradictory. For example, the increases in RFPQ/RFP and DARFPQ/DARFP with the lapse of time mentioned in 3. suggest us the tight binding of quinones to biological materials. Contradictory to the result (iv), RM-AF was found to adsorb covering all of three protein fractions in this experiment. Since the radiochemical method is the most reliable one in this kind of experiment, the adsorption patterns of RFP series may be concluded by this result.

6. Inhibitory effect on the growth of leprosy bacilli in the footpad. A suspension of

Table 1 Inefficacy of ADP and RFPQ on the Growth of Leprosy Bacilli in the Footpad

(Exp. 1.)					
Drug	Drug Content (%)	at 15	17	20	25 weeks
—	—	42*	115	716	238
ADP	0.005	6	39	64	183
"	0.0005	4	42	207	287
RFP	0.005	0	0	0	2
"	0.0005	0	0	3	4
(Exp. 2.)					
		at 15	18	22 weeks	
—	—	83	440	111	
RFPQ	0.0005	48	51	403	
RFP	0.0005	8	11	680	

* Numericals indicate AFB/100 microscopical fields and when they are multiplied by 2.5×10^4 , they indicate all of AFB in each footpad.

leprosy bacilli (Raisl P, $0.6 \times 10^4/0.1$ ml, saline) was subcutaneously injected to each hind footpad of mouse (dd/SPF, male, at 4 weeks after the birth, 12 mice per group). The drugs (ADP, RFP) were dosed for 9–13 weeks. While in another experiment, Raisl P strain of $6.3 \times 10^4/0.05$ ml saline suspension was transmitted to every hind footpad and the drugs (RFPQ, RFP) were dosed for 8–11.5 weeks. In all cases, six footpads out of three mice were pooled and examined microscopically. The results are summarized in Table 1.

It was found that not only ADP but RFPQ is ineffective at this dosage.

7. Discussion. Since RFPQ alone emitted the weak fluorescence ($20 \mu\text{g/ml}$ in CHCl_3 , at 410/520 (nm/nm), 47 units (slit 10/7)) among RFP series, the tracer method or the spectrophotometry⁹⁾ seems to be the most reliable determination method of them. The masking of RFP chromophor (naphthol ring) selectively at its 4-OH by AdCl brought about a marked hypsochromic shift (to 425 nm) and produced ADP which was very liposoluble but unexpectedly very low absorptive derivative of RFP. This poor *in vivo* distribution which was localized even more than DARFP may be caused by its too much high molecular weight. Thus, it was ineffective on leprosy bacilli similarly to RFPQ by mouse level experiment, which distribution was also localized to enterohepatic area alone as well as DARFP. As the result, a series of reactions among RFP or DARFP and carboxylic acid chlorides of higher or lower molecular size could not bring about any preferable result to develop a promising derivative superior to RFP but were merely useful for presuming the probable position of conjugation in RFP skeleton. The result of radiochemical analysis of excreted RFPQ from rat by benzyl alcohol extraction method was published to other report.¹⁰⁾ The important fact was that the enough whole body distribution could be noticed only in 1 hours' radioautogram of RFP group alone and throughout the other groups (metabolite-groups) the poor distributions localized to entero-hepatic area were found even at 1 hour' ones. Similarly to this kind of localized distribution of general macrolide antibiotics including Rifamycins,

the weak point of RFP can not be avoided except very short term *in vivo*. Even though nearly all of usual metabolites of RFP possess their antibacterial effects comparable to RFP itself, this result proves the uselessness of these metabolites including DARFP. Amongst RFP series, RM-AF alone is weakly acidic and all of the others are basic substances. The adsorption of RM-AF very tightly to calf serum suggests us a presumption that the content of RM-AF will gradually increase among metabolites remaining in tissue and organs. While, it is well known fact that p-quinones are stable more than the corresponding p-diphenols and the reduction of the former is more resistant than the oxidation of the latter. For example, the formation of RFP from RFPQ could not be detected when the urinary specimen pooled from mice of RFPQ group was analyzed. In other word, the auto-oxidation of RFP and DARFP to the respective quinones can not be disregarded even under co-existence of biological materials. However, since all of the biological specimens were treated immediately under same condition and as shown on Fig. 3. DARFPQ/DARFP were much higher than the corresponding RFPQ/RFP, DARFP which is more hydrophilic than RFP was thought to be easily oxidable more than RFP in addition to the finding that the contents of quinone-forms gradually increased in gut materials. As for urinary and fecal recovery rate, a specially devised glass apparatus was used and the urinary/urinary+ fecal ratios (%) after 24 hours were 31.5 (RFP), 2.7 (DARFP), 3.5 (RFPQ), and 10.6 (ADP). This fact also indicates the predominant fecal excretion of quinone-forms similar to DARFP. Summarizing these results, it may be suspected that if a quinone of formyl-type is metabolized by demasking of piperazine side residue as in the case of freed RM-AF it may most tightly adsorb on biological materials and remain for long-term *in vivo*. Therefore, the *in vitro* hydrolysis of RFPQ was examined according to the acidic hydrolysis of RFP to produce RM-AF. However, on the resultant chromatogram, a slight spot of RM-AF alone could be detected and most of RFPQ changed into impure materials¹¹⁾. Through analysis of urinary metabolites of RFPQ group, a notable amount of DARFPQ was detected by radiochemical scan of TLC plate when it was developed by n-PrOH-CHCl₃-Dioxane (1:3:1) saturated with 0.1 M phosphate, pH 6.5 and by CHCl₃-Dioxane-MeOH (1:3:1). Therefore, the *in vivo* desmethylation is possible even in the case of quinone form. Contrary to the anxiety about the possible antigenicity of RM-AF and quinone-forms, the immunosuppressive effect has been reported¹²⁻¹⁴⁾. Kasik, *et al.*¹⁵⁾ reported that the main substance which causes immunosuppression is RM-AF when examined by PHA-induced blast formation. Although RM-AF binds probably to polysaccharides which can be associated by its tight binding to dextran sulfate and so the possible non-specific influence must be considered in the case of *in vitro* experiment, the accelerative effect of RFP on the progress of immunological disorders in leprosy patients must not be neglected when remembering the mechanism of action of RFP as an inhibitor of protein chain initiation and elongation through inhibition of RNA polymerase. The other important problem is the side action of RFP on gastro-intestinal tract.

It is well known fact that enterobacteria are living *in vivo* anaerobically¹⁶⁾. Several

authors reported^{16,17)} that RFP and Clindamycin (CLM) are highly effective on bacteroides. CLM is known to be an antibiotics increasing the action area by its *in vivo* desmethylation. It was re-examined by using *Bact. fragilis* ($3.8 \times 10^{4,6}$ microbes) and *Bact. dystasonis* ($2.9 \times 10^{4,6}$ microbes) to each 5 ml of semi-liquid GAM. After incubation at 37°C for 48 hours in Gas-Pak the completely inhibitory molecular concentrations were :

Bact. fragilis (3.8×10^4 , 3.8×10^6 , $\times 10^{-6}M$) ; RFP (6.15, 6.15), CLM (55.5, 55.5), U-24729 A (N-desmethyl pentyl CLM, 500, 500), Homosulfamine (167, 500), B 663 (167, 167), DDS and Ciba 1906 (>500, >500). *Bact. dystasonis* (2.9×10^4 , 2.9×10^6 , $\times 10^{-6}M$) ; RFP (6.15, 18.5), CLM and U-24729 A (6.15 6.15), Homosulfamine (both incomplete at 500), B 663 (55.5, 167), DDS (>500, >500), Ciba 1906 (both incomplete until 167).

This result reminds us of the significance of nutritional state during RFP therapy. RFP was effective even on ophthalmic leprosy¹⁸⁾ and detected even in peripheral nerve¹⁹⁾. However, when considering its weak point as a sort of macrolide-type antibiotics, a rational combined therapy such as alternative form will further be necessary for both preserving drug action constantly and minimizing the dosage.

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Abstract

Metabolism of RFP and of the representative metabolites was individually compared by tracer method.

1. The results were discussed especially concerning the metabolites remaining for long-term *in vivo*.
2. A new derivative of RFP named as ADP was synthesized. Through the similar reaction between RFP or DARFP and aromatic or alicyclic carboxylic acid chloride the presumable position in RFP skeleton of conjugation such as glucuronide conjugation was discussed.
3. *In vivo* inhibitory effect of protein on the oxidation of RFP series to their respective quinone-forms was examined using cysteine as a model of *in vivo* SH residue.
4. The predominant adsorption of RFP series on IgM of calf serum was concluded.
5. The low efficacy of RFPQ and ADP on the growth of leprosy bacilli was concluded by mouse footpad method. The uselessness of DARFP was also presumed.
6. The antibacteroidal effects of RFP was compared with those of some antileprosy drugs and the influence of RFP on *in vivo* enterobacteria was mentioned.
7. The relation between these findings and RFP therapy was discussed.

References

- 1) S. Tsutsumi, M. Gidoh, M. Narita, S. Koide, and T. Funadzu : On characteristic antiinflammatory effects of several antileprosy drugs. The U.S. Japan Cooperative Medical Science Program, the 12th Joint Conference on Leprosy, (1977).
- 2) S. Tsutsumi, Y. Sakamoto, and M. Gidoh : Pharmaceutical view of Rifampicin. *La Lepro*, **43**, 1-7 (1974).
- 3) H. Nakagawa and M. Sunahara : A new Rifampicin metabolite found in human urine. *Rinshō Yakuri*, **3**, 305-307 (1972).
- 4) K. Akimoto, K. Ono, and Y. Nanpo : Absorption, distribution, metabolism, and excretion of Rifampicin (RFP) in the rat. *Jap. J. Antibiotics*, **23**, 250-256 (1970).
- 5) G. Binda, E. Domenichini, A. Gottardi, B. Orlandi, E. Ortellì, B. Pacini, and G. Fowst : RFP, a general view. *Arzneimittelforsch.*, **21**, 1907-1977 (1971).
- 6) S. Tsutsumi, Y. Sakamoto, M. Gidoh, and S. Hazama : Trials for analyzing pigmental antileprosy drugs. The 20th Eastern Meeting of Japan Leprosy Association, (1971).
- 7) H. Nakagawa, M. Machida, and M. Sunahara : On naphthohydroquinone dehydrogenase in human saliva. Proceedings of the symposium on chemical physiology and pathology, **10**, 196-204 (1970).
- 8) H. Nakagawa : On the binding of Rifampicin on serum protein. *Rinshō Yakuri*, **2**, 362-364 (1971).
- 9) H. Nakagawa and M. Sunahara : Metabolism of Rifampicin in the human body (the 1st report). *Kekkaku*, **48**, 167-176 (1973).
- 10) H. Nakagawa, M. Sunahara, and S. Tsutsumi : A hydrophilic metabolite of RFP. *Chemotherapy*, **21**, 1916-1917 (1973).
- 11) S. Tsutsumi, Y. Sakamoto, and M. Gidoh : Acidic hydrolysis of RFPQ as the model of a possible *in vivo* metabolic pathway. *LSM.*, 621 (1974).
- 12) D. C. Graber, J. Jebaily, R. L. Galphin, and E. Doering : Light chain proteinuria and humoral immunoincompetence in tuberculous patients treated with Rifampin. *Amer. Rev. Resp. Dis.*, **107**, 713-717 (1973).
- 13) K. Nessi, R. Pallanza, and G. Fowst : Rifampicin and immunosuppression. *Arzneimittelforsch.*, **24**, 832-836 (1974).
- 14) S. Gupta, M. H. Grieco, and S. Siegel : Suppression of T-lymphocyte rosettes by Rifampicin. Studies in normals and patients with tuberculosis. *Ann. Int. Med.* **82**, 484-488 (1975).
- 15) J. E. Kasik, M. Monick, and J. S. Thompson : Personal Communication.
- 16) Y. Morishita and T. Mitsuoka : Antibody responses in germ-free chickens to bacteria isolated from various sources. *Japan. J. Microbiol.*, **17**, 181-187 (1973).
- 17) S. M. Finegold : Antimicrobial therapy of anaerobic infections. *Chemotherapy* **21**, 1326-1329 (1973).
- 18) Cooperative Research Team on Chemotherapy of Leprosy among National Leprosaria in Japan : Therapeutic effect of RFP on leprosy. I. clinical effect. *La Lepro*, **43**, 8-25 (1974).
- 19) A. C. McDougall, J. A. Rose, and D. G. Graham-Smith : Personal Communication.

Rifampicin (RFP) 代謝の基礎的研究

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RFP ならびにその代表的な代謝物がそれぞれ別個にトレーサー法で代謝を比較された。

1, その結果につき特に体内に永く残留する代謝物に関し討論された。

2, ADP と名付けられる RFP の新誘導体が合成された。似た様な反応が RFP または DARFP と二, 三の芳香属または脂環属カルボン酸クロライドの間でおこなわれ, それを通じて, グルクロン酸抱合の様な抱合が RFP 骨格中どこで起るかが推定され, 討論された。

3, RFP 類がそのキノン体へ酸化される場合に, そ

れへの生体蛋白阻止作用がシステインを体内 SH 残基のモデルとして試験された。

4, RFP 類が主に仔牛血清 IgM へ吸着することが結論された。

5, らい菌増殖への RFP キノンと ADP の阻止作用は足臍法によると弱く, DARFP も有用でないことが推定された。

6, RFP の antibacteroidal 効果が若干の治らい薬と比較され, RFP の腸内細菌への影響が述べられた。

7, これらの所見と RFP 治療の関係が討論された。