BIOCHEMICAL EFFECTS OF FORMYCIN B ON *XANTHOMONAS ORYZAE*

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Formycin B, an inosine analogue, inhibits the incorporation of radioactive adenosine, uridine and thymidine, but none of formic acid, orotic acid and inorganic phosphoric acid into the cellular nucleic acid of *Xanthomonas oryzae*. The biochemical effects are readily reversed when formycin B is removed. Cell-free extract from the organism is entirely insensitive to formycin B in respect to phosphorylation of adenosine and thymidine. In consequence, it is likely that formycin B interferes with some process essential to entry of exogenous nucleosides into the cell. The lethal effect of formycin B on the organism is partially overcame by several nucleosides.

Formycin A and formycin B were isolated from the culture filtrate of *Nocardia interforma*[1,2] and identified as 7-amino-3-(β-D-ribofuranosyl) pyrazolo[4,3-d]pyrimidine and 3-(β-D-ribofuranosyl) pyrazolo[4,3-d]6(H)-7-pyrimidone respectively[3]. They are regarded as an adenosine analogue and an inosine analogue respectively possessing unusual C-riboside linkages. Formycin A exhibits inhibition against several kinds of mammalian neoplastic cells[1], *Xanthomonas oryzae*[1] and viruses[4]. When the 7-amino group of formycin A is protected from deamination activity of target organisms by simultaneous addition of coformycin, an inhibitor of adenosine deaminase which was isolated from the same culture filtrate[5,6], the antibiotic spectrum of formycin A is extended to various kinds of bacteria, and its activities are more pronounced[5,7,8]. In EHRLICH ascites cells, formycin A is phosphorylated to formycin A mono-, di- and triphosphate and there is a good evidence to believe that formycin A triphosphate but not formycin A itself is responsible for its lethal action on the neoplastic cell[9,10]. Since formycin B is as active as formycin A only against *Xanthomonas oryzae*[2] and some viruses[4], it was assumed that formycin B could readily be aminated in these organisms to give formycin A. However, our study revealed that this was not the case and that formycin B itself interfered with purine and pyrimidine neucleoside metabolism in *Xanthomonas oryzae*, probably by blocking entry of exogenous nucleosides into the cell. Data leading to this conclusion will be presented in this paper.

Materials and Methods

A modified KREBS-RINGER phosphate buffer (abbreviated as m-KR), in which phosphate was reduced to 5×10^{-5} M and trishydroxyaminomethane was added at 1×10^{-2} M, was used as the basis of incubation media and for washing cells. The pH was adjusted to 7.6 with HCl at room temperature. Cells of *Xanthomonas oryzae* were harvested from 2-day-

cultured agar slants and washed 3 times by resuspending in cold m-KR. For the study of incorporation of radioactive precursors into intact cells, the washed cells were suspended at 2 % v/v in an m-KR based EAGLE's medium in which leucine content was reduced to 1×10^{-5} M. Other additives such as radioactive precursors and inhibitors were dissolved in 0.50 ml of m-KR and added to 0.50 ml of the above cell suspension in an incubation tube (15×100 mm) which was kept in an ice bath until incubation was initiated. Radioactive precursors were purchased from Dai-ichi Chemical Co., Tokyo, and the following abbreviations are used: ^{3}H-TdR for thymidine-6-(nominally)-^{3}H, ^{3}H-UR for uridine-5-(nominally)-^{3}H, ^{14}C-AR for adenosine-U-^{14}C and ^{32}Pi for ^{32}P-inorganic phosphoric acid. Radioactive precursors were suitably diluted with respective unlabeled precursors so that active incorporation could continue during an incubation period. Incubation was carried out at 27°C on a reciprocating shaker for an indicated period of time, and terminated by the addition of 5 ml of ice-cold 5 % trichloroacetic acid (abbreviated as TCA). The resulting insoluble residue was washed 3 times in cold 5 % TCA, defatted with ethanol-ether mixture (1 : 1, v/v), hydrolyzed with 3 N NH_4OH and placed in a counting vial containing 9 ml of scintillation mixture (PPO and naphthalein in dioxane) for radioactivity measurement in a Beckman Liquid Scintillation System. For the study of nucleoside kinases, cells of *Xanthomonas oryzae* in a late logarithmic growth phase were harvested, washed 3 times in m-KR and ruptured by 3 successive decompression passages at 3,000 psi. By spinning the resulting juice at 10,000 g for 30 minutes in a refrigerated centrifuge, a supernatant was obtained which was used as an enzyme solution. The reaction mixture for enzyme assay contained, in 0.50 ml, 10 μmoles of ATP, 2 μmoles of $MgCl_2$, 2.5 μmoles of NaF, 100 μmoles of Tris-HCl buffer, pH 7.4, 0.10 ml of the above enzyme solution and either 10 mμmoles of ^{14}C-AR (0.05 μc) or 100 μμ moles of ^{3}H-TdR (0.25 μc). To the reaction mixture, 0.1 mcg of coformycin[6] was added for the assay of adenosine kinase, while 100 mcg of bovine serum albumin was added for the assay of thymidine kinase to stabilize the enzyme[11]. Incubation continued for 30 minutes at 27°C. Under these conditions, the nucleotide formation was a linear function of incubation time. The reaction was terminated by heating in a boiling water bath for 3 minutes. After removal of denatured protein by centrifugation, 0.30 ml of the reaction mixture was pippeted onto a 2×2 cm square of DEAE cellulose paper (Toyo Roshi Co., Tokyo) and processed as reported[12]. Antagonism of normal nucleosides and bases to formycin B was studied by the agar plate method: Antagonists were mixed in agar plates (see below) at varying concentrations, and solutions of formycin B were placed in cylinders. The diameters of inhibitory zones on these plates were compared with those on the control plates which had received no antagonists in the agar. Ingredients of the agar plate were glutamic acid, 2.0 (mg/ml); K_2HPO_4, 2.0; $MgCl_2\cdot6H_2O$, 1.0; $FeSO_4\cdot7H_2O$, 0.1; sucrose, 20; yeast extract, 2.0; Polypeptone, 5.0; and powdered agar (Wako Co., Tokyo), 12. They were dissolved in hot distilled water and the solution was neutralized to pH 6.8 and autoclaved. Cells from a 2-day-cultured agar slant were dispersed in the sterilized solution. Ten ml of the cell suspension were placed in a petri dish (10 cm in diameter) to form an agar plate.

Results

Effects of formycin B on the incorporation of various precursors into the cellular macromolecule were studied and compared with those of formycin A and oxoformycin B. The latter compound is believed to be a detoxified metabolite derived from either formycin A or formycin B[13]. As shown in Table 1, formycin B appeared almost identical with formycin A in the every biochemical aspect except for no inhibition of protein synthesis. Incorporation of thymidine, uridine and adenosine were inhibited, whereas those of orotic acid, formic acid and inorganic phosphoric

acid were not affected. The characteristic inhibition of nucleoside uptake could be interpreted as follows: Incorporation of nucleosides into nucleic acids involves several process including nucleoside entry into the cells (a), phosphorylation to the level of nucleoside monophosphate (b), nucleotide interconversion and further phosphorylation to the level of nucleoside triphosphate (c) and polymerization of the triphosphates to form nucleic acids (d). The process (c) and (d) are common to the incorporation process of formic acid as well as of orotic acid. Accordingly, the lesion of formycin B must be localized in early stages of nucleoside metabolism, that is in (a) or (b), but not in the *de novo* synthesis route leading to the level of purine and pyrimidine nucleoside monophosphates nor in any later process as (c) and (d). For step (b) in *Xanthomonas oryzae*, nucleoside kinase but not the combination of nucleoside phosphorylase (or hydrolase) and nucleotide pyrophosphorylase should be responsible, because neither ^{14}C-adenosine nor ^{3}H-thymidine uptake into the acid-insoluble fraction was affected by the addition of 10-time-molar equivalent of the each corresponding base. No direct effect on the energy metabolism is suggested by the lack of effect on protein synthesis. Since the detoxified metabolite, oxoformycin B, failed to show these biochemical effects, it is reasonable to assume that these effects are highly related to the lethal action of formycin B as well as of formycin A on *Xanthomonas oryzae*. Another evidence for the assumption was that none of these effects was demonstrated with *Bacillus subtilis* and EHRLICH ascites tumor cells to which formycin B showed no cytotoxicity.

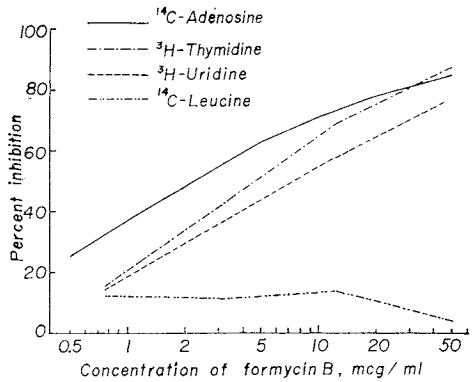
Table 1. Effects on incorporation of radioactive precursors into cellular macromolecules

Inhibitors (10 mcg/ml)	Percent of control which received no inhibitor						
	^{3}H-TdR	^{3}H-UR	^{14}C-AR	^{14}C-6-Orotic acid	^{14}C-Formic acid	^{32}Pi	^{14}C-U-Leucine
Formycin B	44	69	35	110	113	103	108
Formycin A	63	81	30	100	110	100	77
Oxoformycin B	94	121	106	125	103	115	93

Approx. 0.05 μc of each precursor was added per tube. Final concentrations tested were 5×10^{-7} M for thymidine, 1×10^{-5} M for uridine, adenosine and leucine while ^{14}C-6-orotic acid, ^{14}C-formic acid and ^{32}Pi were used without diluting with unlabeled carriers. Incubation continued for 60 minutes. Further analysis of the labeled precipitates according to SCHMIDT-THANNHAUSER procedure revealed that thymidine and uridine were mostly incorporated into DNA and RNA respectively. Radioactivities derived from adenosine, orotic acid, formic acid and Pi became soluble upon heating in 5 % TCA while that from leucine remained insoluble.

Fig. 1. Effects of formycin B at varying concentrations on incorporation of radioactive precursors into cellular macromolecules.

Incubation continued for 60 minutes. Details of the process are as for Table 1.

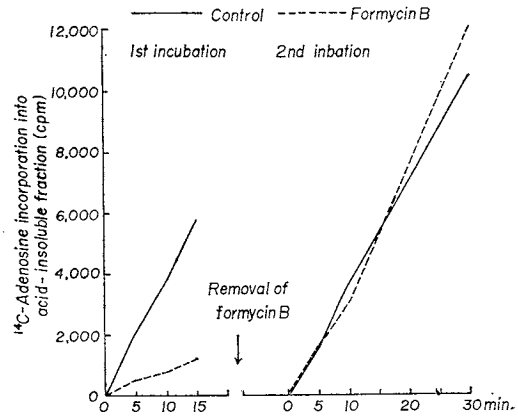


The inhibitory effect of varying concentrations of formycin B on the uptake into the cellular macromolecule of ^{14}C-adenosine, ^{3}H-thymidine, ^{3}H-uridine and ^{14}C-leucine was determined and the results are shown in Fig. 1. It should be noted that the suppression of nucleoside uptake was approximately proportional to the logarithmic concentrations of formycin B while the incorporation of

leucine was not inhibited to any significant extent up to 50 mcg/ml.

To determine whether the inhibition is reversible, the incorporation of ^{14}C-adenosine into a formycin B-inhibited system was measured before and after the removal of formycin B by washing cells. In Fig. 2, one may notice a prompt onset of formycin B effect as well as a complete relief from the inhibition by a brief washing of cells in a formycin B-free medium. At the concentration 10 mcg/ml of formycin B, approximately 80 % inhibition appeared as early as 5 minutes. The degree of inhibition remained constant throughout the period of this experiment and upto 60 minutes in a separate experiment. Similar results were obtained using ^{3}H-thymidine as a precursor. These results led us to assume that formycin B itself but not any converted form such as a nucleotide was involved in the inhibition. If such a lethal synthesis is a prerequisite, the degree of inhibition should be cumulative during the incubation period and furthermore, the inhibitory effect should not be abolished upon washing the cells due to a phosphorylation trap mechanism.

Fig. 2. Relief from the effect of formycin B by changing medium.



First incubation started with 6 tubes of control group (without formycin B) and 6 tubes of formycin B group (with 10 mcg/ml of formycin B). Three tubes of each group were labeled with ^{14}C-adenosine and terminated during the first incubation for radioactivity measurement. After the first incubation, cells in the remaining tubes (that is 3 of control group and 3 of formycin B group; unlabeled) were washed separately in cold m-KR and submitted to the second incubation in the regular incubation medium with ^{14}C-adenosine. Other conditions were as given under Materials and Methods and as for Table 1.

Table 2. Effects of formycin B and formycin A on adenosine kinase and thymidine kinase

Antibiotic		Adenosine kinase	Thymidine kinase
None		100 %	100 %
(mcg/ml)		(9,620 cpm)	(2,580 cpm)
Formycin B	20	124	116
	100	139	127
Formycin A	20	102	129
	100	91	151

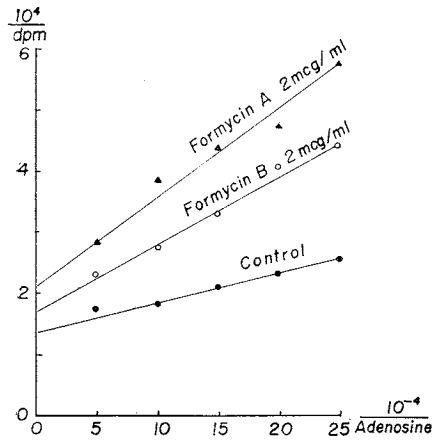
Processes are given under Materials and Methods.

In order to localize the blocking site of the antibiotic more clearly, its possible effect on adenosine kinase and thymidine kinase of *Xanthomonas oryzae* was determined. The rates of nucleotide formation from ^{14}C-adenosine and ^{3}H-thimidine by the cell-free extract are arbitrarily expressed here as adenosine kinase activity and thymidine kinase activity respectively. However, neither nucleosidase nor nucleoside phosphorylase was likely to be involved in these systems because incubation in the presence of 100 mcg/ml of corresponding unlabeled bases, that is adenine for the assay of adenosine kinase and thymine for the assay of thymidine kinase, showed no dilution of radioactivity in the nucleotide fractions. As shown in Table 2, no significant inhibition by either formycin B or formycin A up to 100 mcg/ml was observed, or rather, they appeared more or less stimulatory in these systems. Formycin A was reported to be a good substrate for adenosine kinase from several

Fig. 3. LINEWEAVE-BURK plot of the effect of formycin B and formycin A on the incorporation of ^{14}C-adenosine (a) and ^{3}H-thymidine (b) into the cold TCA-insoluble fraction.

Incubation continued for 30 minutes at 27°C, during which the incorporation of ^{14}C-adenosine as well as ^{3}H-thymidine into the cold TCA-insoluble fraction was a linear function of incubation period. Other conditions were as given under Materials and Methods and as for Table 1.

(a)



(b)

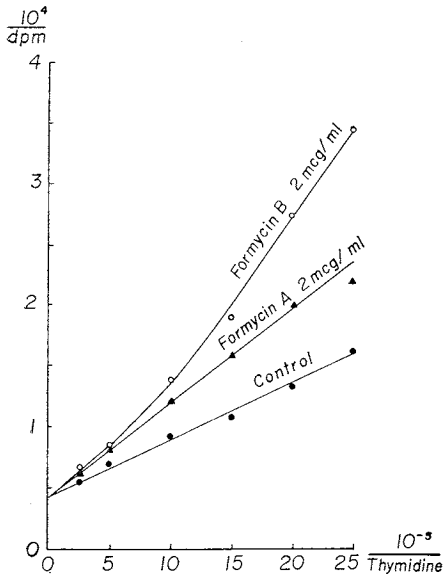


Table 3. MICHAELIS-MENTEN kinetic constants for the incorporation of nucleosides into the cold TCA-insoluble fraction and for the inhibition by formycin B or formycin A

Substrate	Km	Ki	
		Formycin B	Formycin A
Adenosine	3.8×10^{-6}	2.2×10^{-5}	1.1×10^{-5}
Thymidine	1.4×10^{-6}	1.9×10^{-5}	4.7×10^{-5}

Table 4. Protection by normal metabolites against the lethal effect of formycin B and formycin A

Antagonist (100 mcg/ml)	Percent protection	
	Formycin B	Formycin A
Adenosine	70	80
Guanosine	30	neg
Inosine	75	60
Thymidine	70	40
Uridine	70	40
Inosine (50 mcg/ml) + Uridine (50 mcg/ml)	40	—
Adenine	neg	30
Hypoxanthine	neg	70
Thymine	neg	50
Uracil	neg	40

These values were obtained by the same type of experiment as for Fig. 4. The values represent the averages of tripricate culture.
neg : negligible protection.

sources[14,15,16], and therefore, it could inhibit the reaction by competing for the enzyme. However, the relative magnitudes of Km values for adenosine and for formycin A would explain the above result. Thus, it is likely that these antibiotics interfere with some process before nucleoside kinases participate.

To get a better understanding of the character of the biochemical effects on intact cells, a kinetic study was attempted in which the external concentration of ^{14}C-adenosine was varied in the presence of a constant concentration of formycin B or formycin A. As shown in Fig. 3-a, it was observed that formycin B as well as formycin A noncompetitively inhibited adenosine incorporation into the TCA-insoluble fraction. A similar type of experiment was conducted with regard to ^{3}H-thymidine as a substrate, and the results are shown in Fig. 3-b. The inhibition pattern of formycin A with respect to thymidine appeared competitive, whereas the plot of the

formycin B system showed a curve coming to a common intercept with that of control on the ordinate. Kinetic parameters for the incorporation of purine or pyrimidine nucleosides into the TCA-insoluble fraction and its inhibition by formycin B or formycin A are given in Table 3.

It should be noticed in Fig. 3 that the reaction velocity in every interrupted system increases in parallel with the concentration of a substrate, even though the infinite amount of a substrate, in the case of adenosine, would be unable to abolish completely the inhibition. Assuming the blockage in the process of nucleoside uptake is fatal to the cell, at least partial antagonism to the lethal effect of formycin B by excess amount of any of those nucleosides would be expected. This possibility was investigated by the agar plate method. Results are shown in Fig. 4 and Table 4. Both the purine and pyrimidine nucleosides did overcome the lethal effect of formycin B to some extent, whereas the nucleic acid bases were entirely ineffective. Combination of uridine and inosine was not so effective as each of them alone. An antagonism spectrum to formycin A was somewhat different from that to formycin B, especially in the significant protection by the bases, which was an unexpected finding. The magnitude of protection by the normal metabolites was found to decrease upon prolonged incubation. This phenomenon may be due to higher stability of formycin B than those of antagonists to the catabolic action of the target organism.

The assumption that formycin B itself was responsible for the biochemical effects presented so far was further ascertained by determining its possible conversion when

Fig. 4. Antagonism between formycin B and adenosine with respect to cell growth of *Xanthomonas oryzae* in an agar plate.

Processes are given under Materials and Methods. Inhibition zone were measured after 24-hour incubation. Calculation of % protection was as follows: The size of the inhibition zone of the antagonized system at 20 mcg/ml of formycin B* is equivalent to that of control at 6 mcg/ml of formycin B**.

$$\text{Percent protection} = \left(1 - \frac{6}{20}\right) \times 100\ \% = 70\ \%$$

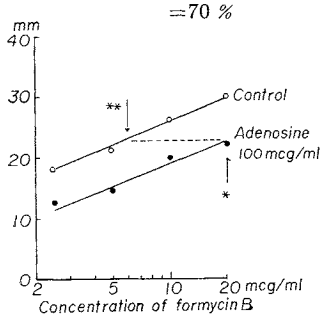


Table 5. Metabolic conversion of formycin B by *Xanthomonas oryzae*

Medium and washing		Cellular fractions	
		acid soluble	acid insoluble
Formycin B	>90 %		
Formycin A	0.4 %	nucleoside 0.2	0.01
Oxoformycin B	5 %	nucleotide 0.3	

A wet cell-pellet of 0.33 ml was dispersed in 10 ml of regular incubation medium and incubated with 500 mcg (5.8×10^6 dpm) of ^{3}H-formycin B at 27°C for 3 hours on a reciprocating shaker. Incubation was terminated by chilling. Cells were spun down and washed free of the radioactive medium. The medium and washings were combined and submitted to analysis of formycin B, formycin A and oxoformycin B by the procedures including adsorption and elution from a charcoal column, high voltage paper electrophoresis and paper chromatography as reported[8]. Cells were extracted with cold 10 % perchloric acid. After removal of potassium perchlorate, the extract was submitted to analysis of nucleotides formed by the procedures including adsorption and elution from a charcoal column and DEAE-cellulose paper chromatography developed with 50 mM ammonium formate and 1 M formic acid. Nucleosides, mostly unreacted formycin B, were readily washed off DEAE-cellulose paper with 1 mM ammonium formate before or after chromatography. Cold-perchloric acid-insoluble residue was processed as given under Materials and Methods for radioactive measurement. A sample of formycin B was kindly tritiated by Dr. C. KOMAI, National Institute of Health, Tokyo.

incubated with the target organism. As shown in Table 5, formycin B appeared stable upon extensive incubation in a thick cell-suspension of *Xanthomonas oryzae*. More than 90 % of the original amount of formycin B remained intact in the medium and washings. Radioactivities which moved with carrier formycin A and oxoformycin B in paper electrophoresis and paper chromatography were not more than 0.4 % and 5 % respectively of the original amount of ^{3}H-formycin B. Radioactivity found in the acid-insoluble fraction was not sufficient to allow further analysis, although it was significant compared with the value of unincubated control. In a separate experiment where formycin B was exposed to an actively growing culture of *Xanthomonas oryzae* for 24 hours, formation of formycin A, which was identified by radioautography, was still limited to only 3 % of the original amount. Under such conditions, approximately one third of the formycin B added was recovered as oxoformycin B, whereas the rest remained intact.

Discussions

PATERSON and SIMPSON[17] reported that nucleoside analogues such as 6-methylthioinosine inhibited various aspects of nucleoside metabolism in intact erythrocytes. As a possible mechanism of action, some interference with entry of nucleosides into the cell was proposed because those analogues failed to exert any effect on a disrupted cell system. A similar mechanism of action is suspected for the effect of formycin B, although detailed biochemical events which might be involved in the entry of nucleosides into the cell are still obscure. Since *Xanthomonas oryzae* dose retain the *de novo* synthesis pathway of purine and pyrimidine nucleotides, it is difficult at this moment to explain why the blockage in the entry of exogenous nucleosides could be fatal to the organism. It may be an answer that the capacity of the *de novo* pathway of this organism is not large enough to sustain active propagation. PARKS *et al.*[18] observed that formycin B was a potent competitive inhibitor of erythrocytic purine nucleoside phosphorylase. However, no such enzyme inhibition seems responsible for the formycin B effect against *Xanthomonas oryzae*. Recently, SKEHEL *et al.* reported[19] that 2-mercapto-1-(β-4-pyridoethyl) benzimidazole, which has antiviral activity and might be regarded as a nucleoside analogue, inhibited in a competitive manner the incorporation of radioactive uridine into the nucleotide pool of chick embryo cells, though the exact locus of its lesion was not established. Considering the potent antiviral activity of formycin B, it should be of great interest to find among nucleoside analogues a possible correlation between the inhibitory effect on the nucleoside metabolism and antiviral effect. It is likely that formycin A works in a more complicated fashion than formycin B does, for instance, for its inhibition of protein synthesis, although their effects are similar in several aspects.

References

1) HORI, M.; E. ITO, T. TAKITA, G. KOYAMA, T. TAKEUCHI & H. UMEZAWA: A new antibiotic, formycin. J. Antibiotics, Ser. A 17: 96~99, 1964.
2) KOYAMA, G. & H. UMEZAWA: Formycin B and its relation to formycin. J. Antibiotics, Ser. A 18: 175~177, 1965
3) KOYAMA, G.; K. MAEDA, H. UMEZAWA & Y. IITAKA: The structural studies of formycin and formycin B. Tetrahedron Letters 6: 597~602, 1966
4) TAKEUCHI, T.; J. IWANAGA, T. AOYAGI & H. UMEZAWA: Antiviral effect of formycin and formycin B. J. Antibiotics, Ser. A 19: 286~287, 1966
5) NIIDA, T.; T. NIWA, T. TSURUOKA, N. EZAKI, T. SHOMURA & H. UMEZAWA: Isolation and characteristics of coformycin. The 153rd Scientific Meeting of Japan Antibiotics Research Association, Jan. 27, 1967.

6) SAWA, T.; Y. FUKAGAWA, I. HOMMA, T. TAKEUCHI & H. UMEZAWA: Mode of inhibition of coformycin on adenosine deaminase. J. Antibiotics, Ser. A 20: 227~231, 1967
7) SAWA, T.; Y. FUKAGAWA, I. HOMMA, T. TAKEUCHI & H. UMEZAWA: Formycin-deaminating activity of microorganisms. J. Antibiotics, Ser. A 20: 317~321, 1967.
8) UMEZAWA, H.; T. SAWA, Y. FUKAGAWA, I. HOMMA, M. ISHIZUKA & T. TAKEUCHI: Studies on formycin and formycin B in cells of EHRLICH carcinoma and *E. coli*. J. Antibiotics, Ser. A 20: 308~316, 1967
9) CALDWELL, I. C.; J. F. HENDERSON & A.R.P. PATERSON: Resistance to purine ribonucleoside analogues in an ascites tumor. Canadian J. Biochem. 45: 735~744, 1967
10) HENDERSON, J. F.; A.R.P. PATERSON, I. C. CALDWELL & M. HORI: Biochemical effects of formycin, an adenosine analog. Cancer Res. 27: 715~719, 1967
11) OKAZAKI, R. & A. KORNBERG: Deoxythmidine kinase of *E. coli*. J. Biol. Chem. 239: 269~274, 1964
12) KIM, J. H.; A. S. GELBARD, A. G. PEREZ & M. L. EIDINOFF: Effect of 5-bromodeoxyuridine on nucleic acid and protein synthesis and viability in HeLa cells. Biochim. Biophys. Acta 134: 388~394, 1967
13) ISHIZUKA, M.; T. SAWA, G. KOYAMA, T. TAKEUCHI & H. UMEZAWA: Metabolism of formycin and formycin B *in vivo*. J. Antibiotics 21: 1~4, 1968.
14) SCHNEBLI, H. P.; D. L. HILL & L. L. BENNET, Jr.: Purification and properties of adenosine kinase from human tumor cells of type H. Ep. No. 2 J. Biol. Chem. 242: 1997~2004, 1967
15) ACS, G.; D. C. WARD, A. CERAMI, E. REICH & S. URETSKY: Metabolism and functional properties of formycin nucleotides and polynucleotides. 7 th International Congress of Biochemistry, B-121, 1967
16) KLENOW, H.: Personal communication.
17) PATERSON, A.R.P. & A. I. SIMPSON: Inhibition of ribonucleoside metabolism in EHRLICH ascites tumor cells by purine analogue ribonucleosides. Canadian J. Biochem. 43: 1701~1710, 1965
18) SHEEN, M. R.; B. K. KIM & R. E. PARKS, Jr.: Purine nucleoside phosphorylase from human erythrocytes. III. Inhibition by the inosine analogue, formycin B, of the isolated enzyme and of nucleoside metabolism in intact erythrocytes and sarcoma 180 cells. J. Biol. Chem. (submitted)
19) SKEHEL, J. J.; A. J. HAY, D. C. BURKE & L. N. CARTWRIGHT: Effects of actinomycin D and 2-mercapto-1-(β-4-pyridoethyl) benzimidazole on the incorporation of ^{3}H-uridine by chick embryo cells. Biochim. Biophys. Acta 142: 430~439, 1967.