23. Minoru Kawashima, Yoshio Hamada, and Shizuko Fujii: Studies on Acidomycin. VI. Metabolic Antagonism with Biotin*.

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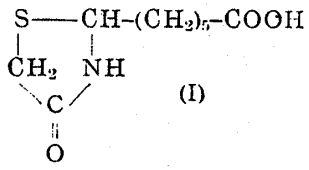
The new antibiotic substance, acidomycin, was isolated from the culture broth of *Streptomyces acidomyceticus* by Ogata in our laboratory[1]. Miyake, *et al.*[2] studied the chemical structure of acidomycin and confirmed that it was identical with the compound, 2-(5-carboxypentyl)-4-thiazolidone (I), reported recently by the Pfizer's group[3,4].



(I)

This compound was also isolated independently in Abbott laboratory[5] and Lederle laboratory[6], and was named "actithiazic acid" by the former and "mycobacidin" by the latter.

One of the authors, Hamada[7], observed that rabbit urine, after the intramuscular injection of acidomycin, gave a double zone during the assay procedures by the cylinder plate method using *Mycobacterium tubeculosis* var. *avium*. The double zone was composed of an outside zone owing to the inhibitory effect of acidomycin and an inside zone formed by the stimulation of unknown factor.

It was reported that this thiazolidone antibiotic was very effective *in vitro* against *M. tuberculosis*[2,3,5], whilst inffective *in vivo*[5]. It seemed that these two findings were correlated to each other closely, and first we had considered that these phenomena were induced by antagonism between acidomycin and its own biological degradation products.

The authors, therefore, studied the antagonism between acidomycin and its own chemical degradation products in the test for several organisms in a biotin-deficient medium by inhibition analysis. For the structural resemblance, biotin was also used as sample for these experiments.

Further studies by means of paper partition chromatographic analysis of the rabbit urine after injection of acidomycin (200 γ/g. body weight), normal urine and pure biotin

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1) Ogata, *et al.*: Studies on Streptomyces and its Antibiotics. IX. One Product exhibiting Antitubercular Activity. Presented at the 17th Meeting of the Kinki Branch of Japan Antibiotics Association held in Hiroshima in Sept. 1951. In press; "Studies on Acidomycin. II. On the Situation of Acidomycin-Producing Strains on the Taxanomy and its Mutants obtained by Ultraviolet Irradiation"; "Studies on Acidomycin. III. On the Cultivation of Acidomycin-Producing Strains." In press: These were reported at the 63rd Meeting of Japan Antibiotics Resarch Association held at National Institute of Health, Tokyo, on September 26, 1952.

2) Miyake, *et al.*: "A New Antibiotic, Acidomycin; Its Isolation and Chemical Structure," presented at the Regular Meeting of Kinki Branch of the Pharmaceutical Society of Japan, held in Osaka on September 20, 1952.

3) B. A. Sobin: J. Am. Chem. Soc., **74**, 2947 (1952).

4) W. M. McLamore: *Ibid.*, **74**, 2946 (1952).

5) W. E. Grundy: Antibiotics and Chemotherapy, **2**, 399 (1952).

6) E. Tejera, *et al.*: Antibiotics and Chemotherapy, **2**, 333 (1952).

7) Y. Hamada, *et al.*: "Studies on Acidomycin. V. Anti-acidomycin Eeffect Observed by Cylinder Plate Method in Rabbit Urine." Presented at the 64th Meeting of Japan Antibiotics Research Association, Tokyo, November 14, 1952.

solution indicated that the anti-acidomycin factors in both urine have the same pooled Rf value. This value was identical with that of biotin. Therefore, it seemed probable that one of the anti-acidomycin factors which was increased in the urine after intramuscular injection of acidomycin might be biotin.

Methods

Inhibition analysis—The organism employed in these studies were *Lactobacillus casei*, *Lactobacillus arabinosus* 17~5 and a strain of *Mycobacterium tuberculosis* var. *avium*. The media used were varied with the particular organism investigated. All, however, were completely synthetic in composition and biotin-free (Tables I and II). The culture media were used in 5 cc. per tube. Each inoculum was precultivated in several transfers in a biotin-free medium shown in Tables I and II. Bacterial growth was inhibited by the addition of acidomycin sodium to the medium. The acidomycin concentration used was 8, 40, and 150 γ/cc. medium for *M. tuberculosis*, *L. arabinosus*, and *L. casei*, respectively. Pimelic acid, thioglycollic acid, and ω-acetaminoheptanoic acid were added to each series by 1,000 γ/cc. in the initial tube containing 5 cc. of the medium and then the contents of tubes further diluted by consecutive 5-fold dilution. In the case of the biotin, it was added in 2 γ or 20 mγ per cc. in the initial tube. Each series were sterilized by autoclaving at 15 lbs. for 10 minutes and, after inoculation, incubated at 37° in the case of *M. tuberculosis* and *L. casei*, and at 30° for *L. arabinosus*. After incubation (48 hrs. for *M. tuberculosis*, 24 hrs. for *L. casei* and *L. arabinosus*), the resulting turbidity was measured by electrophotometry.

For estimating the reversibility of the urine, 1 cc. of each urine sample was added to 5 cc. of the medium containing 40 γ/cc. of acidomycin, the contents of tubes further diluted by consecutive 5-fold diluting, and then inoculated with *L. arabinosus*. After 24 hrs' incubation the reversibility of the urine samples was measured electrophotometrically.

Chromatography—Paper partition chromatography was used for identification of anti-acidomycin factor in the urine samples. Chromatograms were run at a room temperature (15°) by the ascending and descending methods with butanol-water and phenol-water as a mobile phase, respectively, and the running time was normally 20 hrs. After developing, the paper strip was dried in the air and then divided into 10 pieces between the origin and the front. Each piece was eluted with 5 cc. of distilled water with frequent shaking, 1 cc. of the eluate was added into the tubes containing the medium for biotin assay (Table II), and the resulting mixture was inoculated with *L. arabinosus* and then incubated for 24 hrs. at 30°. The resulting turbidity was measured by electrophotometry.

TABLE I Medium for *M. tuberculosis*

Na_2HPO_4	3.0 g.	Asparagine	5.0 g.
KH_2PO_4	4.0 g.	Glycerol	20.0 g.
$MgSO_4$	0.6 g.	Tween-80 (2.5% solution)	20.0 cc.
Sodium citrate	2.5 g.	Distilled water	1,000.0. (pH 7.0)

TABLE II Medium for *L. casei* and *L. arabinosus*

Hydrolyzed casein	250 mg. as total nitrogen	Thiamine	100 γ
Glucose	4.0 g.	Riboflavine	100 γ
Sodium acetate	1.0 g.	Pyridoxine	200 γ
Cystine	40.0 mg.	Niacine	100 γ
Tryptophan	40.0 mg.	Ca Pantothenate	100 γ
Adenine	1.0 mg.	Solution A*	1.0 cc.
Guanine	1.0 mg.	Solution B**	2.0 cc.
Uracil	1.0 mg.	Sufficient water to make	100 cc. (Double
PABA	30 γ	strength)	

* Dissolve 25 g. of monobasic potassium phosphate and 25 g. of dibasic potassium phosphate in sufficient water to make 250 cc. of solution.

** Dissolve 10 g. of $MgSO_4$, 0.5 g. of NaCl, 0.5 g. of $FeSO_4$, and 0.5 g. of $MnSO_4$ in sufficient water to make 250 cc.

Results

In the inhibition analyses as shown in Figs. 1~3, acidomycin was antagonized by biotin in all cases, and the antagonistic ratio was 2×10^{-3}, 4×10^{-7}, and 1×10^{-7} for *M. tuberculosis*, *L. arabinosus*, and *L. casei*, respectively. Pimelic acid, thioglycollic acid, and ω-acetaminoheptanoic acid had no effect on the inhibitory activity of acidomycin.

Our studies showed that biotin was the sole antagonist of acidomycin with high antagonistic ratio among the substances related to the degradation products of acidomycin tested.

We also studied the reversibility of rabbit urine (normal, 0~8 hrs, and 8~24 hrs. after injection) against inhibitory effect of acidomycin with *L. arabinosus*. As shown in Fig. 4, the reversibility of the urine was increased after injection, and it was found that the biotin contents determined by microbiological assay technique with *L. arabinosus* were 40 mγ/cc. of urine in normal and 200 mγ/cc. after injection.

In chromatographic analysis, as shown in Figs. 5~6, the maximum turbidity appeared in the pooled Rf values of 0.8~0.9 and 0.1~0.2 in butanol-water and phenol-water system, respectively. Control biotin also had the same Rf value. This was in accordance with the data obtained by Lichstein and Christman[8], who stated that biotin moved to the top of the chromatogram having Rf value approximately 0.9 with phenol-water system.

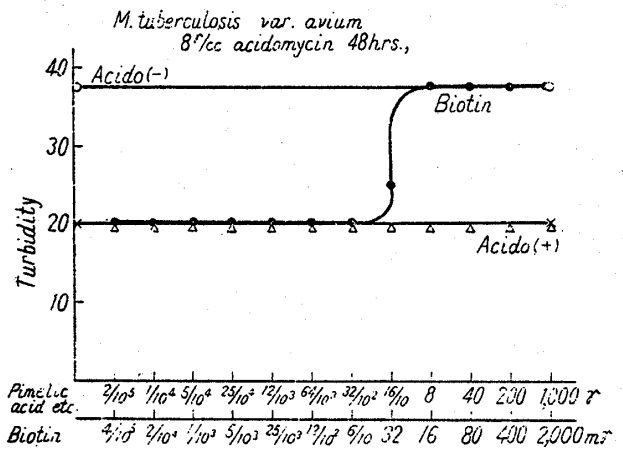

Fig. 1

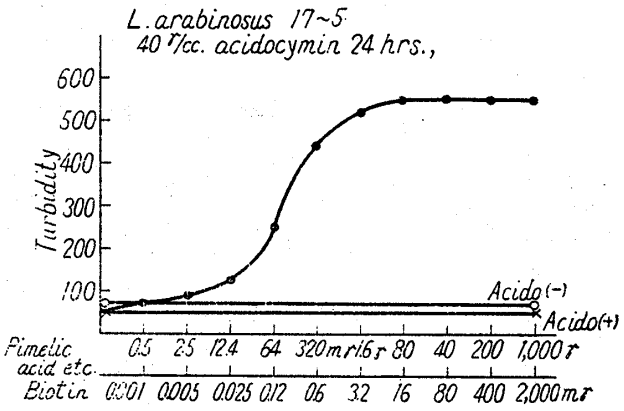

Fig. 2

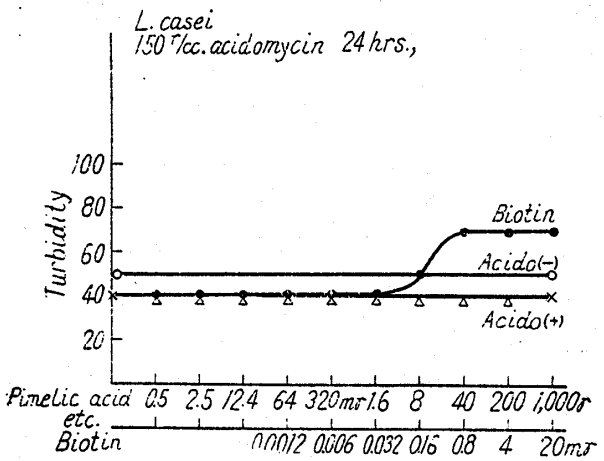

Fig. 3

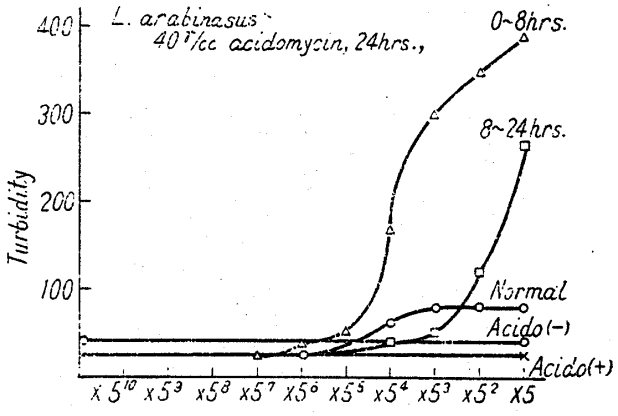

Fig. 4

8) H. C. Lichstein, J. F. Christman: J. Bact., 58, 565 (1949).

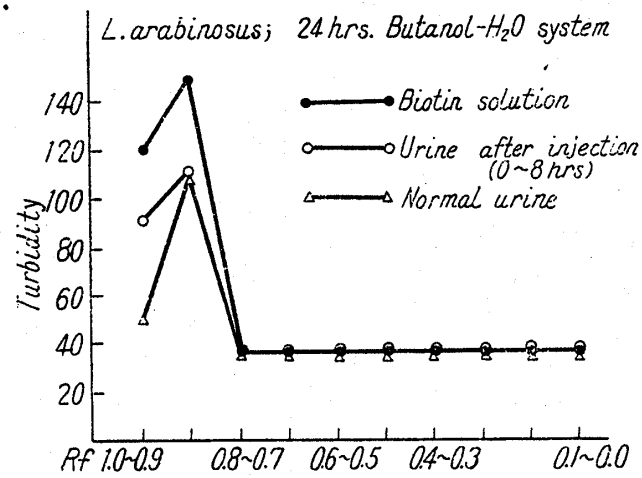

Fig. 5

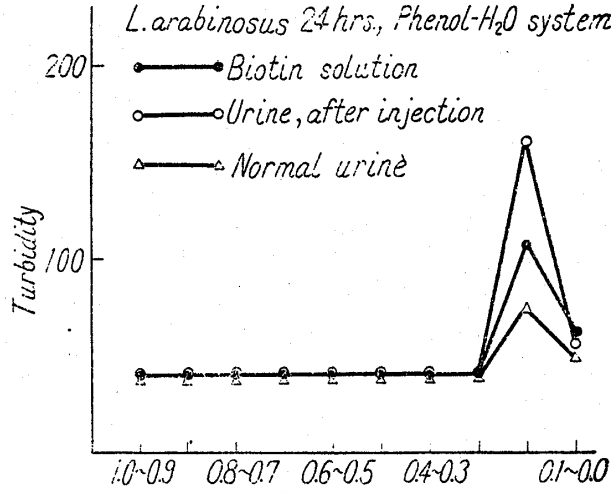

Fig. 6

Conclusion

We have concluded that one of the anti-acidomycin factors in rabbit urine may be biotin from the following facts ;

1). Acidomycin was antagonized by biotin in a highly antagonistic ratio against *M. tuberculosis*, *L. casei*, and *L. arabinosus* in synthetic biotin-deficient medium.

2). The reversibility of urine against inhibitory action of acidomycin was increased after injection.

3). It seemed that the reversibilities of urine run in parallel with the biotin contents of urine.

4). Paper partition chromatographic analysis of urine indicated that one of the anti-acidomycin factors in rabbit urine had the same pooled Rf value as that of biotin.

5). One of the authors, Hamada[7], succeeded in the reconstruction of the double zone in cylinder plate method by filling the cup with a mixture of biotin and acidomycin, reproducibly.

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Summary

The authors studied the antagonism between acidomycin (thiazolidone antibiotic) and its own chemical degradation products, namely, pimelic acid, thioglycollic acid, and ω-acetaminoheptanonic acid, against *L. arabinosus*, *L. casei*, and *M. tuberculosis* var. *avium* in biotin-deficient medium by inhibition analysis. Biotin was also used as a sample of this experiments. Biotin was the sole antagonist of acidomycin among the substances related to acidomycin. Chromatographic analysis showed that one of the anti-acidomycin factors in rabbit urine might be biotin.

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