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14. Minoru Kawashima, Akira Miyake, Teruji Hemmi, and Shizuko Fujii : Biochemical Studies on Acidomycin. IV. Avidin-combinability of Acidomycin and Its Related Compounds.*

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The authors recently reported that intramuscular injection of acidomycin sodium into normal rabbits increaed the biotin-excretion in their urine.[1,2] As to the cause of this phenomenon, the authors presumed that biotin was liberated from the biotin-protein complex *in vivo* by the administration of acidomycin through intramuscular route and increased the biotin level in urine.

This report describes a model experiment designed to obtain some insight into interaction between acidomycin or its related compounds and a biotin-protein complex *in vitro*. As protein avidin, a component of egg white capable of combining specifically with biotin, was employed and the effect of acidomycin or its related compounds on avidin-biotin complex (A. B-complex) was observed.

Materials and Methods

Microörganism used in this experiment was *Lactobacillus arabinosus* 17-5 (ATCC 8014; IFO 3070), and the basal medium employed was completely synthetic in composition.[1] The composition of the basal medium was as follows :

Hydrolyzed casein	1.0 g.
Glucose	4.0 〃
Sodium acetate	1.0 〃
Cystine	40 mg.
Tryptophan	40 〃
Adenine, guanine, uracil, xanthine	2.0 〃 each
p-Aminobenzoic acid	30 γ
Thiamine hydrochloride	100 〃
Riboflavin	100 〃
Pyridoxine hydrochloride	200 〃
Niacine	100 〃
Ca pantothenate	100 〃
Solution A*	1.0 ml.
Solution B**	1.0 〃
Sufficient water to make	100 cc. (double strength).

* 25 g. each of KH_2PO_4 and of K_2HPO_4 dissolved in sufficient water to make 250 cc. of solution.

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

Samples tested were : (I) Acidomycin sodium, (II) acidomycin hydrazide, (III) acidomycin esters (methyl and ethyl), (IV) 2-phenyl-3-benzylthiazolidone, (V) 2-phenylthiazolidone-4, and (VI) 2,3-diphenylthiazolidone.

Compounds (I) to (III) possessed antitubercular activity, while (IV) to (VI) affected the growth slightly. In studies on their ability to combine, amount of acidomycin and its related compounds up to 1,000 mγ/cc. was employed, since the previous experiment showed that *L. arabinosus* had no influence in an amount of less than 30 γ/cc. of acidomycin sodium.[2]

Avidin : Avidin was prepared from egg white according to the method described by Eakin, Snell, and Williams.[3] To a strained egg white (10 eggs, approximately 350 cc.) is added 2 volumes

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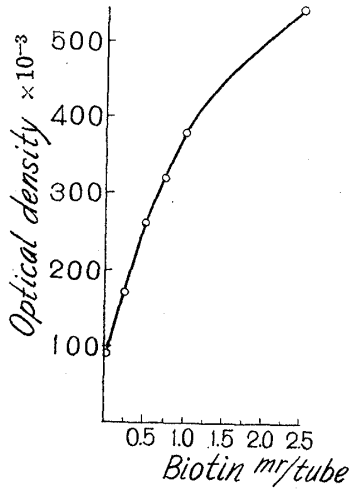
1) M. Kawashima, Y. Hamada, S. Fujii : This Bulletin, **1**, 94 (1953).
2) M. Kawashima, S. Fujii : *Ibid.*, **1**, 328 (1953).
3) L. D. Wright, H. R. Skeggs : Proc. Soc. Exptl. Biol. Med., **64**, 150 (1947).

of cold acetone, and the mixture is stirred until the protein has completely coagulated. The coagulum is filtered through a cheese-cloth bag and squeezed dry by hand. It is broken up, washed by suspending in 1 L. of distilled water for 1 hr, and then separated from the washing as described above for the acetone solution. The washings containing no avidin are discarded.

The crude washed product is extracted by suspending in 200 cc. of 2% $(NH_4)_2SO_4$ solution for several hours (or over night), and the extract is then filtered as described before. The extraction is repeated with an additional 200 cc. of 2% salt solution. The two extracts are combined, 50 g. of $(NH_4)_2SO_4$ is added to each 100 cc. of the solution at room temperature, and the solution stirred until the salt is completely dissolved. When the solution is saturated with $(NH_4)_2SO_4$, the precipitate is collected by centrifugation.

The precipitate is dissolved in 20 cc. of distilled water and then dialyzed against running tap water for 48 hrs. The dialyzed solution is lyophilized, yielding 1.0 g. of solid crude avidin.

Microbiological Experiments—The methods employed in this study were those commonly used in microbiological assay with lactic acid bacteria. Biotin was determined microbiologically with *L. arabinosus.*[2] The extent of bacterial growth was determined turbidimetrically after 24 hrs. with Coleman Junior spectrophotometer at 650 mμ. Avidin was used in several solutions containing 200~10 γ/cc. Sterilization of avidin solution was effected by filtration through a Berkefeld filter. Combinability studies were carried out directly in the microbiological assay medium prior to seeding with *L.arabinosus.*[4] In the determination of avidin combinability the response of



Eig. 1. Response of *L. arabinosus* to Biotin (*L. arabinosus* 30°, 18 hrs.)

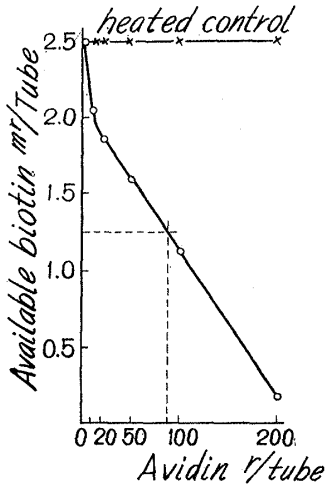


Fig. 2. Effect of Avidin on Growth of *L. arabinosus* (Media supplemented with 2.5 mγ/tube of biotin)

Table I.

Tube No.	Biotin (mγ/tube)	Avidin (γ/tube)	Optical density ×10³
1	0		90
2	0.25		170
3	0.50		260
4	0.75		320
5	1.00		380
6	2.50		540
7	〃	10	520
8	〃	20	510
9	〃	50	480
10	〃	100	420
11	〃	200	150
7′	〃	10	540
8′	〃	20	〃
9′	〃	50	〃
10′	〃	100	530
11′	〃	200	540

Sterilized in the customary manner by steaming for 5 mins. at 15 lbs. after avidin was added.

4) L. D. Wright, H. R. Skeggs: Arch. Biochem. Biophys., **12**, 27 (1947).

L. arabinosus to biotin is first determined (Fig. 1). Secondly, the activity of the avidin preparation in combining with biotin is measured by the relative growth of *L. arabinosus* following the aseptical addition of varying amounts of avidin to previously sterilized tubes containing a constant amount of biotin (Fig. 2 and Table I). Finally, the relative affinity of avidin to biotin and acidomycin or its related compounds is determined by relative growth of *L. arabinosus* following the addition of avidin to the mixture of biotin and acidomycin or its related compounds (Table II).

The standard curve is used to determine the amount of biotin uncombined with avidin, and thus, indirectly the amount of acidomycin or its analogs taking part in the reaction.

Table II.

Tube No.	Biotin (mγ/tube)	Avidin (γ/tube)	Analogs (mγ/tube)		Optical density ×10³
0	—				70
1	0.25				180
2	0.50				260
3	0.75				330
4	1.00				380
5	2.50				610
6	〃	200			70
7	〃	100			410
8	〃	50			530
9	〃	20			570
10	〃	10			590
11	〃	200	Acidomycin	1000	70
12	〃	〃	〃	100	65
13	〃	〃	〃	20	68
14	〃	〃	〃	2	〃
15	〃	〃	Ethyl ester	1000	〃
16	〃	〃	〃	100	65
17	〃	〃	〃	20	70
18	〃	〃	〃	2	68
19	〃	〃	Hydrazide	1000	70
20	〃	〃	〃	100	〃
21	〃	〃	〃	20	68
22	〃	〃	〃	2	70
23	〃	〃	2-Phenyl-3-benzylthiazolidone	1000	68
24	〃	〃	〃	100	70
25	〃	〃	〃	20	〃
26	〃	〃	〃	2	69
27	〃	〃	2-Phenylthiazolidone	1000	69
28	〃	〃	〃	100	65
29	〃	〃	〃	20	70
30	〃	〃	〃	2	65
31	〃	〃	2,3-Diphenylthiazolidone	1000	68
32	〃	〃	〃	100	70
33	〃	〃	〃	20	〃
34	〃	〃	〃	2	72

Results and Discussion

The most interesting bound form of biotin is its combination product with a special protein-like constituent of egg white called avidin. Avidin readily combines with biotin *in vitro* simply on mixing their solutions and the avidin-biotin complex (A. B-complex) thus formed is incapable of being utilized by *L. arabinosus* as biotin source.

It was demonstrated that the crude avidin preparation employed in this investigation could combine with one-half its amount of biotin by 88 γ of avidin at 2.5 mγ/5 cc. of biotin level (Fig. 2).

Acidomycin and its related compounds failed to liberate biotin from A.B-complex in concentrations not more than 1,000 mγ/5 cc., whether the preparation has anti-

tubercular activity or not (Table II).

It has been shown that acidomycin and its related compounds having antitubercular activity were antagonistic to biotin. Evidence that acidomycin and its analogs failed to liberate biotin from A.B-complex, irrespective of whether or not they have antitubercular activity, indicates the unavailability of A.B-complex as an experimental model to elucidate the mode of action of acidomycin in increasing urinary biotin excretion.

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Summary

The action of acidomycin and its analogs on avidin, as the experimental model of the biotin-protein complex, during the formation of avidin-biotin complex (A.B-complex) was examined *in vitro*. It was found that these compounds failed to free biotin estimated by *L. arabinosus* method against inactivation by avidin. It was concluded that A.B-complex cannot be employed as a model of experiment designed to obtain some answers for the increasing urinary biotin excretion in acidomycin-injected rabbits.

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