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Affinity of Avidin for Certain Analogs of Biotin.

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Wright and Skeggs recently have described a procedure whereby the relative affinity of avidin for biotin and for biotin analogs possessing no microbiological activity readily may be compared.[1] The method was illustrated by a study of the relative affinity of avidin for the available spatial isomers of biotin.

This paper presents additional data on the avidin combinability of certain compounds having some structural similarity to biotin. The compounds studied (I-XIX) may be classified into 4 types: (1) acyclic analogs of desthiobiotin differing from desthiobiotin and its homologs by an opening of the imidazolidone ring (II-VII),[2,*] (2) hydantoin derivatives differing from desthiobiotin homologs by the replacement of the methyl group with an oxygen atom (VIII,IX),* (3) "model" compounds (X-XIII) bearing some relationship to the original pyrimidine structure proposed and later retracted by Kögl *et al.* for α-biotin,[3,4,*] (4) aromatic and cyclic aliphatic derivatives with a urea ring or a urea ring with an ω-carboxy aliphatic side chain (XIV-XIX).[5,†]

The procedure employed[1] involved the addition of avidin to mixtures of biotin and the analog under investigation. The biotin and avidin were used in stoichiometric amounts and the amount of analog varied. In the case of compounds having definite avidin combinability an amount of biotin is available for the growth of *Lactobacillus arabinosus* that is equivalent to the amount of analog taken up by the avidin. The "relative affinity" is then expressed arbitrarily as the ratio of the concentration of analog to biotin at which one-half of the biotin remains free and available for growth of the test organism. It is obvious that the ratio will be low for analogs for which avidin has considerable affinity and high for those analogs that do not combine readily with avidin.

None of the compounds studied had any activity in lieu of biotin for promoting growth of *L. arabinosus*. Similarly, none of the compounds studied, *in the amounts employed,*

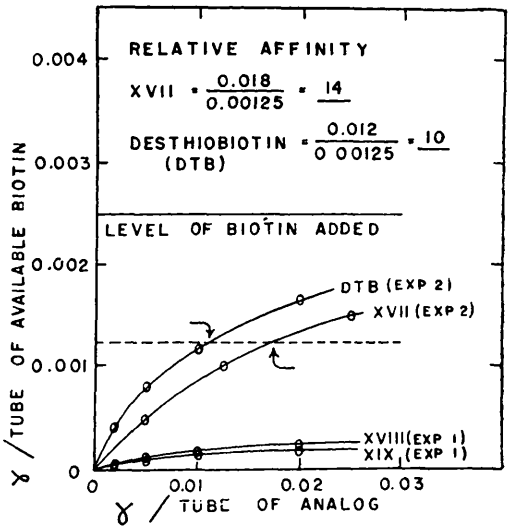


FIG. 1.
The relative affinity of avidin for biotin and biotin analogs.

[1] Wright, L. D., and Skeggs, H. R., *Arch. Biochem.*, 1947, **12**, 27.

[2] Schultz, E. M., in press.

* We are indebted to Dr. E. M. Schultz of these laboratories for compounds II through XIII.

[3] Kögl, F., Verbeek, J. H., Erxleben, H., and Borg, W. A. J., *Z. physiol. Chem.*, 1943, **279**, 121.

[4] Kögl, F., and Borg, W. A. J., *Z. physiol. Chem.*, 1944, **281**, 65.

[5] English, J. P., Clapp, R. C., Cole, Q. P., Halverstad, I. F., Lampen, J. O., and Roblin, R. O., Jr., *J. Am. Chem. Soc.*, 1945, **67**, 295.

† These compounds were supplied through the courtesy of Dr. J. O. Lampen of the Research Laboratories of the American Cyanamid Company.

TABLE I.
The Affinity of Avidin for Certain Analogs of Biotin.

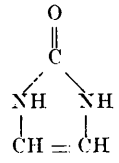
Exp. No.	Biotin, γ	Analog, γ	Avidin, γ	Turbidity
1	0			84
	.00025			141
	.0005			176
	.00075			200
	.0010			220
	.0015			275
	.0025			318
	.0025		20	300
	.0025		50	250
	.0025		100	86
	.0025		200	83
	.0025	.002 XVIII	100	99
	.0025	.005 "	100	106
	.0025	.010 "	100	128
	.0025	.020 "	100	139
	.0025	.002 XIX	100	93
	.0025	.005 "	100	111
	.0025	.010 "	100	125
	.0025	.020 "	100	130
2	0			97
	.00025			163
	.0005			204
	.00075			246
	.0010			260
	.0015			300
	.0025			350
	.0025		20	335
	.0025		50	284
	.0025		100	122
	.0025		200	95
	.0025	.0050 XVII	100	212
	.0025	.0125 "	100	262
	.0025	.0250 "	100	300
	.0025	.050 "	100	330
	.0025	.002 DTB*	100	189
	.0025	.005 "	100	244
	.0025	.010 "	100	276
	.0025	.020 "	100	310

* *dl*-Desthiobiotin.

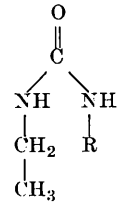
had significant antibiotin activity for the assay organism.

Combinability studies were carried out directly in the microbiologic assay medium by the aseptic addition of avidin just prior to seeding with *L. arabinosus*.

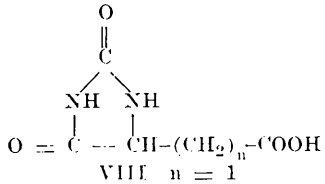
Of the compounds studied only No. XVII, XVIII, and XIX showed definite avidin combinability. Compound No. XVII, which differs from biotin in the replacement of the sulfur atom by a dimethylene group, had an affinity ratio of approximately 14. For reference *d,l*-epi-allobiotin has an affinity ratio of about 3-6[1] while *d,l*-desthiobiotin has an affinity ratio of approximately 10 (Table I, Fig. 1). The specificity of the avidin combinability reaction further is illustrated by the fact that Compound XIX in which the ureylene group is attached to the cyclohexane ring at positions 3 and 4 rather than 2 and 3 with respect to the valeric acid side chain, although showing detectable avidin combinability (Table I, Fig. 1), had an affinity ratio too high for practical measurement. Compound XVIII in which the ureylene group is attached 3,4 to a cyclohexyl ring



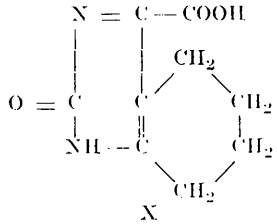
I



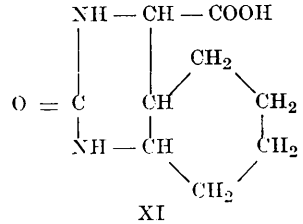
II R = $-CH_2-CH_2-CH_2-CH_3$
III R = $-CH_2-CH_2-CH_2-CH_2-CH_2-COOH$
IV R = $-CH_2-CH_2-CH_2-CH_2-CH_2-CONH_2$
V R = $-CH_2-CH_2-CH_2-CH_2-CH_2-COOC_2H_5$
VI R = $-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-COOH$
VII R = $-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-COOC_2H_5$



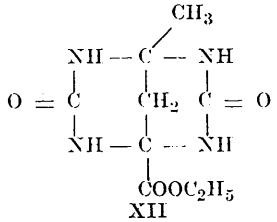
VIII n = 1
IX n = 2



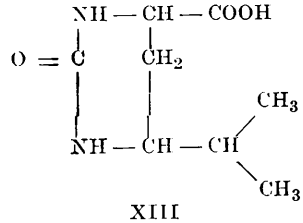
X



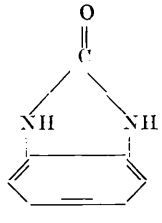
XI



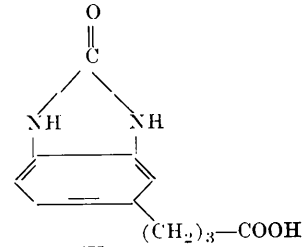
XII



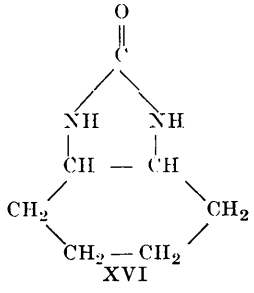
XIII



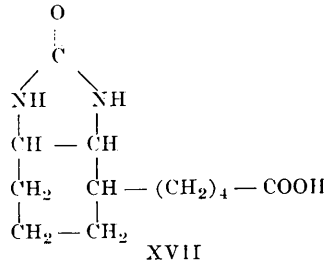
XIV



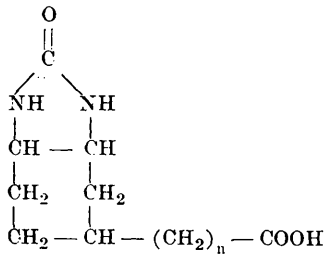
XV



XVI



XVII



XVIII n = 3
XIX n = 4

containing a butyric acid side chain similarly showed definite avidin combinability but had an affinity ratio too high for practical measurement.

The failure of avidin to show significant affinity for the acyclic analogs of desthiobiotin (II-VII) is not unexpected. Opening of the imidazolidone ring would alter considerably the spatial arrangement of the molecule.

The inactivity of the hydantoin derivatives probably is attributable either to the shortness of the side chain in the compounds studied or to the existence of the ring predominantly in an enol form showing little resemblance to the cyclic urea ring of biotin.

While the resemblance of some of the pyrimidine derivatives studied to the original α-biotin formula of Kögl *et al.* is quite remote, Compound XIII is similar to the corresponding desthio derivative. The data do not permit a conclusion as to whether the failure of avidin to show significant affinity for this compound is due to the presence of a pyrimidine rather than an imidazolidone ring or to the absence of an ω-carboxy aliphatic side chain of suitable length.

Winnick, Hofmann, Pilgrim and Axelrod[6] have studied the inhibition of certain oxybiotin derivatives with avidin. It was demonstrated that *dl*-oxybiotin, *dl*-oxybiotin methyl ester and hexahydro-2-oxo-1-furo-(3,4)-imidazole-4-pentanol (the alcohol corresponding to oxybiotin) combined with avidin. Approximately one unit of avidin was required to inactivate each compound for *Saccharomyces cerevisiae*. Cis-3,4-Diamino-2-tetrahydrofuranvaleric acid (oxybiotin diamino carboxylic acid) in which the cyclic urea ring is absent failed to combine with avidin. The procedures employed by Winnick *et al.* are capable of a qualitative interpretation but do not permit a conclusion as to the relative affinity of avidin for biotin in comparison with the affinity for the compounds studied.

Summary. The avidin combinability of a number of compounds having some structural similarity to biotin was investigated. The specificity of the avidin reaction was emphasized by the finding that of the 19 compounds studied avidin possessed significant affinity only for δ-(2,3-ureylene-cyclohexyl)-valeric acid (XVII), γ-(3,4-ureylene-cyclohexyl)-butyric acid (XVIII) and δ-(3,4-ureylene-cyclohexyl)-valeric acid (XIX). The relative affinity ratio for Compound XVII was found to be about 14. Compounds XVIII and XIX, although definitely capable of combining with avidin, had affinity ratios too high for practical measurement.

[6] Winnick, T., Hofmann, K., Pilgrim, F. J., and Axelrod, A. E., *J. Biol. Chem.*, 1945, **161**, 405.