atmosphere of air. The rate of deamination of all of them was determined in the same experiment on each homogenate preparation.

The results of a representative experiment are plotted in Fig. 1. Here it is seen that tyramine is rapidly deaminated in accordance with previous findings.[5] All the compounds are oxidized, but "Marfanil" is oxidized at the slowest rate. However, the slowness with which this oxidation occurs *in vitro* is not necessarily an indication of its rate of detoxication in the body, since phenethylamine which is inactive as a pressor agent when taken orally is only slowly deaminated by this system under these conditions.

From these experiments one may conclude that "Marfanil" and certain related compounds containing an aliphatic primary amino group are oxidized under conditions wherein that action is attributed to amine oxidase. It is possible that these findings account at least in part for the usual lack of as satisfactory a systemic response to "Marfanil" when it is administered orally or parenterally as has been reported to result from its topical application.

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THE RELATIONSHIP OF LYSOZYME, BIOTIN AND AVIDIN[1]

ON the basis of common source and certain similarities in chemical and biological properties, Laurence[2] has suggested that lysozyme is identical with biotin-saturated avidin. Meyer[3] found that biotin stimulates lysozyme activity when it is added to preparations containing lysozyme and avidin. Both authors have called attention to the association of avidin activity with lysozyme activity in various concentrates of avidin or lysozyme[3] and have discussed the possible identity or close relationship of these substances. A convenient method for the isolation and crystallization of lysozyme has been reported recently from this laboratory.[4] With pure lysozyme preparations available, we have investigated the relationship suggested by Laurence's and by Meyer's work.

We have been unable to obtain a stimulation of lytic activity by addition of crystalline biotin to pure or impure lysozyme preparations. In repeated trials with various lysozyme preparations and with raw egg white, biotin had no detectable effect on lytic activity as measured by the assay method of Boasson.[5] This method depends on the quantitative photometric measurement of the rate of lysis of phenol-killed *Micrococcus lysodeikticus* and is accurate to approximately 10 per cent. Numerous variables were investigated, including (1) the use of synthetic biotin (Merck) and isolated natural biotin (free acid, S.M.A. Co.); (2) ratios ranging from 10 to 1000γ of biotin per mg of lysozyme; (3) preliminary incubation at room temperature and at 37° C. of solutions of lysozyme plus biotin for periods ranging from 10 minutes to 18 hours; and (4) the use of live cells of *M. lysodeikticus*. An attempt was also made to achieve greater sensitivity by permitting both phenol-killed and live cells of *M. lysodeikticus* to lyse for 3 hours at 37°. In no case was increased lytic activity observed on the addition of biotin.

Similarly, no increase in lysis was observed when biotin was added to avidin preparations. This was true in both the presence and absence of lysozyme (Table 1, preparations 4 and 5 compared with 6). We also considered the possibility that avidin might inactivate lysozyme by combining with it and that this effect might be counteracted by the addition of biotin. However, when the lysozyme-free avidin (preparation 6) was added to an equal amount (by weight) of lysozyme (preparation 1), no repression of the lytic activity of the lysozyme took place. Subsequent addition of varying amounts of biotin to the mixture again resulted in no change in lytic activity. No evidence was obtained from any of these experiments that either avidin or biotin is involved in the lytic activity generally ascribed to lysozyme.[6]

The biotin content of our pure lysozyme is inconsistent with the hypothesis that biotin acts as a prosthetic group in lysozyme. The results of biotin assays of three lysozyme preparations, given in Table 1, are similar to those reported by Williams, Schlenk and Eppright[7] for purified proteins and enzymes (*i.e.*, trypsin, chymotrypsin, renin, insulin, casein, tobacco-mosaic virus). The purest lysozyme preparation contained only 0.009 ppm. of biotin. A 1:1 stoichiometric combination of biotin with lysozyme would re-

[1] From the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[2] William L. Laurence, SCIENCE, 99: 392, 1944.

[3] William L. Laurence (SCIENCE, 99: 392, 1944) reported an avidin content of 100 units per gram in a six-year-old sample of lysozyme obtained from Karl Meyer (SCIENCE, 99: 391, 1944). D. W. Woolley and L. G. Longsworth (*Jour. Biol. Chem.*, 142: 285, 1942) reported that 1 mg of pure avidin is able to bind 5γ of biotin, *i.e.*, 5,000 units of biotin per gm of avidin; accordingly, Laurence's assay corresponds to about 2 per cent. of free avidin as an impurity in the sample of lysozyme.

[4] Gordon Alderton, W. H. Ward and H. L. Fevold, *Jour. Biol. Chem.*, in press.

[5] E. H. Boasson, *Jour. Immunol.*, 34: 281, 1938.

[6] After the experiments described above had been completed, we obtained 4 avidin concentrates through the courtesy of Dr. Vincent du Vigneaud. These preparations contained approximately 150, 500, 1,000 and 2,500 units of avidin activity per gram according to Dr. du Vigneaud. By E. H. Boasson's (*Jour. Immunol.*, 34: 281, 1938) method, the lysozyme contents were found to be approximately 0.5, 0.5, 0.1 and 14 per cent., respectively. The addition of biotin (270 ppm.) had no influence on the lytic activity of these preparations.

[7] R. J. Williams, F. Schlenk and M. A. Eppright, *Jour. Am. Chem. Soc.*, 66: 896, 1944.

TABLE 1

AVIDIN, BIOTIN AND LYSOZYME CONTENTS OF VARIOUS PREPARATIONS

Preparation	No.	Description	Lysozyme*	Biotin†	Avidin‡		
					Free avidin	Biotin-avidin	Total avidin
			Per cent.	γ per gm	Per cent.	Per cent.	Per cent.
Lysozyme	1	By adsorption method §; isoelectrically precipitated: crystallized once from 5 per cent. NaCl–0.2 M acetate at pH 4.5	100	0.009	0.0014	0.0002	0.0016
Lysozyme	2	By adsorption method §; not isoelectrically precipitated; crystallized twice as above and lyophilized	100	0.040	0.0036	0.0008	0.0044
Lysozyme	3	By adsorption method §; not isoelectrically precipitated; crystallized twice	100	0.12	0.0022	0.0024	0.0046
Crude Avidin Concentrate	4	By-product of lysozyme preparation by adsorption method §	16	7	2.50	0.14	2.64
Crude Avidin Concentrate	5	Preparation No. 4 further purified with ammonium sulfate as described below¶	28	38	13.2	0.76	14.0
Avidin Concentrate	6	By an unpublished adsorption method¶	< 0.1	226	82.0	4.5	86.5

* Determined by the method of Boasson (*Jour. Immunol.*, 34: 281, 1938) and expressed in terms of crystalline lysozyme prepared by the method of Alderton, Ward and Fevold (*Jour. Biol. Chem.*, in press) as the standard.

† Biotin was determined with *Saccharomyces cerevisiae* (F.B.) by the method of Snell, Eakin and Williams (*Jour. Am. Chem. Soc.*, 62: 175, 1940) as modified by Hertz (*Proc. Soc. Exp. Biol. and Med.*, 52: 15, 1943). Lysozyme was refluxed for 2 hours in 6*N* HCl to ensure complete liberation of the biotin. Added biotin was recovered quantitatively; only 10 per cent. of the added biotin was destroyed during 6 hours of hydrolysis. Biotin was freed in avidin concentrates by autoclaving at 118° C. for 1 hour at neutrality. The biotin-binding power of avidin was not always destroyed completely by the short periods of steaming at pH 4.0 in the biotin-assay method.

‡ Free avidin was measured by its biotin inactivating effect; biotin-avidin was calculated from an assay of the biotin liberated by steaming; total avidin is the sum of free avidin and biotin-avidin. Woolley and Longsworth's (*Jour. Biol. Chem.*, 142: 285, 1942) factor of 5γ of biotin per mg of avidin for the biotin-binding capacity of free avidin was used to calculate units of avidin activity to a percentage basis.

§ Gordon Alderton, W. H. Ward, H. L. Fevold, *Jour. Biol. Chem.* In press.

¶ This avidin concentrate was prepared from raw egg white by adsorption on bentonite, followed by washing with dilute potassium chloride solution and elution with alkaline phosphate buffer. The crude concentrate obtained in this way was dialyzed and further purified (after concentration by lyophilization) by solution in 2*M* ammonium sulfate and precipitation by 3.6*M* ammonium sulfate as suggested by Woolley and Longsworth (*Jour. Biol. Chem.*, 142: 285, 1942).

quire a biotin content of about 1 per cent., *i.e.*, about 10^6 times the amount actually present in the purest lysozyme preparation. Like biotin, avidin was found to be present in the lysozyme preparations only in very small amounts. All three lysozyme preparations had equal lytic activity.

A factor that may or may not have been different in our experiments, as compared with those of Meyer,[8] was the biotin content of the test organisms, and the biotin thus introduced in the assay method. Our *M. lysodeikticus* organisms contained about 0.93γ of biotin per gram of dried cells.[9] The amount of biotin introduced by the organisms would therefore be small. If, however, lytic activity involves avidin and biotin, some lysis would result when the test organism is added to avidin; such lysis did not result with our purest avidin preparation. Lysozyme was the only pure substance that lysed the test organisms.

The presence of both lysozyme and avidin in relatively impure concentrates would not be unexpected, in view of the basic nature of both of these proteins. The isoelectric point for lysozyme is about pH 10.8,[4] and that for avidin is about pH 10.0.[10] The hypothesis that the two are not necessarily related, either in biological activity or in chemical identity, is supported by several facts. Woolley and Longsworth[10] obtained highly purified avidin, which they found to be without lysozyme activity. By a different method we also have obtained avidin preparations that are essentially free of lysozyme. On the other hand, lysozyme prepared by the method of Alderton, Ward and Fevold[4] is essentially free of avidin.[11] The molecular weights, electrophoretic mobilities and solubilities of the two pure substances effectively argue against their identity. The facts presented in this paper offer no support to the hypothesis that they are related in biological activity.

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8 Karl Meyer, SCIENCE, 99: 391, 1944.

9 In these assays the biotin was freed for the microbiological assay (see footnote †, Table 1) by being refluxed in 4N HCl for 2 hours on an oil bath; less than 5 per cent. of the biotin present was liberated by steaming at pH 4.0.

10 D. W. Woolley and L. G. Longsworth, *Jour. Biol. Chem.*, 142: 285, 1942.

11 Avidin assays were made directly on the lysozyme preparations, since at pH 4 in the presence of sufficient biotin even 0.1 per cent. of lysozyme was not inhibitory to *Saccharomyces cerevisiae*. Confirmatory evidence of the low avidin content of these lysozyme preparations was obtained from balance studies on the distribution of avidin, biotin and lysozyme. Lysozyme represents about 3 per cent. of the raw egg white proteins (Gordon Alderton, W. H. Ward and H. L. Fevold, *Jour. Biol. Chem.*, in press); avidin, only about 0.06 per cent. Further experimental observations on the disposition of avidin during the isolation of lysozyme led to an upper limit of 0.2 per cent. avidin impurity in our lysozyme preparations.