to see that the society should give its active aid towards a better public understanding of what is going on. The joint project between the State of New York and our society for the post-war establishment of a conservation exhibit in the Zoological Park can well serve as a springboard for the educational services which we hope to accomplish in this most important field. At the risk of reiteration, allow me to say, in somewhat different terms, that the preservation of wild animal life not only in America but in other parts of the world depends upon preserving the habitats and living places in which animal life can be expected to survive. We hear a lot of talk these days to the effect that if our soils run out the food chemists will step in and feed us on synthetic foods and vitamins. This is a questionable possibility, and even though it could be accomplished, life would indeed be tasteless and colorless. In any case, we can not go around feeding vitamins to wild animals. Consequently, from now on this institution intends to do everything in its power to contribute to the preservation of forests and soils both here and in other countries upon which man and wild animal life alike must depend for their future existence.

SPECIAL ARTICLES

THE DEAMINATION OF "MARFANIL" AND RELATED COMPOUNDS[1]

CONSIDERABLE interest has been manifested recently both abroad[2] and in this country[3] in the use of "Marfanil" homosulfanilamide as a topical adjuvant in the treatment of gas gangrene. The general consideration of the compound (p-amino-methylbenzenesulfonamide, first synthesized by Miller, Sprague, Kissinger and McBurney[4]) as a "sulfa" drug has tended to cloud a proper consideration of its action and inactivation.

Recently we included "Marfanil" in a study of the relationship of structure to deamination of a series of substituted benzyl and phenylethylamines by amine oxidase. Because of the timeliness of the study with regard to interest in the compound, the fact that it is relatively ineffective when administered by mouth but is active when used topically, and since Evans, Fuller and Walker[2] have postulated very recently that some such mechanism plays a part in the inactivation of "Marfanil" in the body we have thought it worth while to publish a note on our results as they pertain to this compound.

The procedure was essentially that reported by Beyer.[5] The Warburg respirometer was used for the measurement of the oxygen taken up in the course of the deamination of the compounds. Guinea pig liver homogenates were the source of amine oxidase. The tests were conducted in the presence of cyanide using M/23 or M/16 concentrations of the amines before their dilution by other contents of the flasks. The compounds tested were tyramine, benzylamine, p-sulfamilphenylethylamine and "Marfanil." Each vessel contained 1.5 cc 25 per cent. guinea pig liver homogenate in M/8 phosphate buffer, pH 7.2; 0.1 cc M/15 NaCN; 0.9 cc M/4 phosphate buffer, pH 7.2. The center well contained 0.2 cc 20 per cent. KOH plus 0.1 cc M/15 NaCN. The substrates were placed in the side arms. The reaction was run at 37° C in an

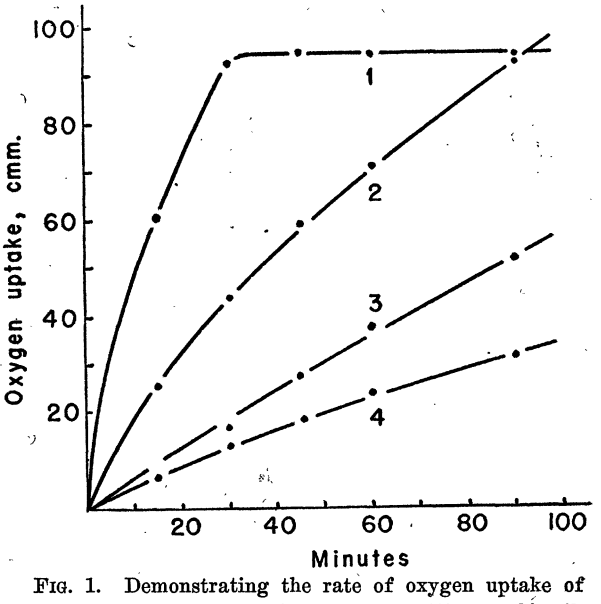

FIG. 1. Demonstrating the rate of oxygen uptake of (1) tyramine hydrochloride, M/23; (2) p-sulfamilphenylethylamine hydrochloride, M/16; (3) benzylamine hydrochloride, M/16; (4) "Marfanil" hydrochloride, M/16. 0.2 cc of each substrate was used.

[1] From the Department of Pharmacology, The Medical-Research Division, Sharp and Dohme, Inc., Glenolden, Pa.

[2] (a) J. Klarar, *Klin. Wschr.*, 20: 1250, 1941. (b) A. Fleming, Special report on tests with Marfanil prepared for Medical Research Council, 1943. (c) C. N. Robinson, *Lancet*, 2: 351, 1943. (d) G. A. G. Mitchell, W. S. Rees and C. N. Robinson, *Lancet*, 1: 627, 1944. (e) D. G. Evans, A. T. Fuller and J. Walker, *Lancet*, 2: 523, 1944. (f) G. Domagk, *Klin. Wschr.*, 21: 448, 1942; *Dtsch. Med. Wschr.*, 69: 379, 1943.

[3] (a) E. A. Bliss and H. C. Deitz, *Jour. Bacteriol.*, 47: 449, 1944. (b) C. A. Lawrence, *Jour. Bacteriol.*, 47: 452, 1944. (c) E. A. Bliss and H. C. Deitz, *Bull. Johns Hopkins Hosp.*, 75: 1, 1944. (d) C. M. McKee, D. M. Hamre and G. W. Rake, *Proc. Soc. Exp. Biol. and Med.*, 54: 211, 1943. (e) D. M. Hamre, H. A. Walker, W. B. Dunham, H. V. van Dyke and G. Rake, *Proc. Soc. Exp. Biol. and Med.*, 55: 170, 1944.

[4] E. Miller, J. M. Sprague, L. W. Kissinger and L. F. McBurney, *Jour. Am. Chem. Soc.*, 62: 2099, 1940.

[5] K. H. Beyer, *Jour. Pharmacol.*, 71: 151, 1941.

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.

KARL H. BEYER
WM. M. GOVIER

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.