Catatorulin Effect of Aneurin Disulphide

ANEURIN disulphide[1], formed by opening of the thiazole ring and oxidation to the —S—S— form, does not give the thiochrome reaction, unless suitably reduced by cysteine; it was reported to have some 60 per cent of the biological activity of aneurin, when given orally to animals. I have found in catatorulin tests, by methods previously described[2], with the deficient pigeon brain that it is at least as active as aneurin (Table 1).

TABLE 1. CATATORULIN TEST WITH BREI, FROM AVITAMINOUS PIGEON BRAIN. SUBSTRATE, SODIUM PYRUVATE

Oxygen uptake $\mu\lambda$/gm./hr. for respiration period 30–120 min.

		Change
No addition	733	—
Aneurin, 0·5 γ	1352	+ 619
Aneurin, 0·25 γ	1122	+ 389
Aneurin disulphide, 0·5 γ	1524	+ 791

TABLE 2. REACTIVATION OF OXIDIZED COCARBOXYLASE FOR CARBON DIOXIDE PRODUCTION. $\mu\lambda CO_2$ PRODUCED IN 15 MIN. FROM SODIUM PYRUVATE IN PRESENCE OF ALKALINE WASHED YEAST, ANEURIN AND MAGNESIUM 28° C.

Addition	$\mu\lambda CO_2$
Nil	31
+ Cysteine, 4 mgm.	48
Aneurin disulp. pyrophos. (1·5 γ)	35
,, ,, ,, + cysteine, 2 mgm. ..	255
,, ,, ,, + cysteine, 4 mgm. ..	311
,, ,, ,, + cysteine, 10 mgm. ..	348
,, ,, ,, + glutathione, 4 mgm. ..	197
,, ,, ,, + B.A.L., 1 mgm. ..	226
,, ,, ,, + cysteine-ester, 4 mgm. ..	91

I have also found that preparations of aneurin disulphide pyrophosphate made by the method of K. Myrback, I. Vallin and I. Magnell[3] by oxidation of cocarboxylase with iodine, when tested by the method of Ochoa and Peters[4] show little or no activity in the decarboxylation of pyruvate by washed yeast; this has been also stated recently by P. Karrer and M. Viscontini[5], so that I can confirm it independently. I have also found that —SH compounds reactivate the preparation for carbon dioxide production, when added immediately after the washed yeast (Table 2); 6 mgm. cysteine hydrochloride per respiration bottle produces a maximum effect (22 mM.); cystine was practically without effect. Myrback *et al.* treated their preparations of aneurin disulphide pyrophosphate with cysteine to reactivate for the thiochrome reaction. As judged by reactivation with cysteine for decarboxylation and restoration of the thiochrome reaction, most of my preparations of this substance were relatively inactive, showing not more than about 10 per cent of the original activity. Upon this basis, catatorulin tests with the dispersion from the avitaminous brain showed an activity corresponding to the amount of aneurin disulphide pyrophosphate present. Since the latter is itself inactive in the yeast test, it is logical to think that it must first be reduced to aneurin before it is active in the catatorulin tests by the —SH compounds present. The fact, however, that *in vitro* these brain enzyme preparations can carry out this change appears still to leave room for the suggestion of Williams and Zima that the —S—S— form of aneurin may play its part in the dehydrogenation.

Synthetic preparations of cocarboxylase (as used here) have been found in this laboratory to give some 60 per cent of the full effect of Lohman and Schuster's cocarboxylase[6]; one possible reason for this is the presence of some oxidized cocarboxylase; since this would be reactivated by cysteine, it is interesting to note that the addition of cysteine to our control synthetic cocarboxylase gives increases of approximately 50 per cent in the carbon dioxide production over the first 10-min. period. L. D. Greenberg and J. F. Rinehart[7] reported an activation of cocarboxylase tests by cysteine.

I am indebted to Messrs. Merck and Hofman le Roche for specimens of cocarboxylase and to the latter for the aneurin disulphide. I am also grateful to R. W. Wakelin for assistance with the tests.

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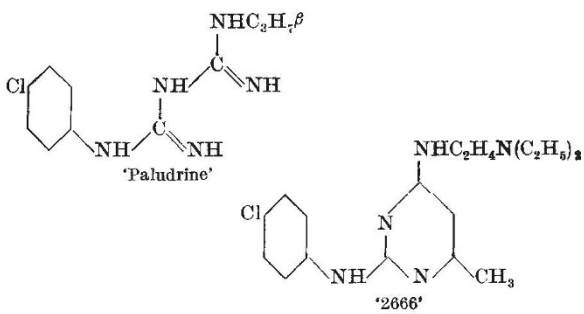
A Possible Mode of Action of 'Paludrine'

IN the evolution of the antimalarial drug 'Paludrine', the diguanide system was selected because it provided structural features similar to those found in the earlier active pyrimidine compound '2666'[1]. The biochemistry of the former drug, together with the results obtained in both experimental and clinical therapy, indicate, however, that it is biologically distinct from the prototype molecule. Thus, for example, therapeutic potency is many times greater, and is apparent not only against the erythrocytic but also against the exo-erythrocytic forms of the malaria parasite. Further, 'Paludrine' does not show the antagonism for riboflavine exhibited by '2666' (and mepacrine) with respect to the growth of the *Lactobacillus casei*, an effect that we associate with the formal structural resemblance of the latter drugs to the vitamin, and which may also be connected with their parasiticidal activity.



So far, the biochemical and biological researches of our colleagues, Drs. Madinaveita and Davey, have not provided any explanation for these facts. We now suggest, on the basis of certain chemical observations, that the antimalarial activity of 'Paludrine' may be connected in some way with an interference with the porphyrin metabolism or enzyme systems of the parasite. 'Paludrine' forms a copper derivative the analysis of which gives one atom of copper combined with two molecules of the drug. Assuming a symmetrical disposition of the diguanide molecules in a planar structure (compare phthalocyanine), space models indicate the arrangement formulated below. The methyl groups printed in italic type are accommodated either above or below the general plane of the complex, and are therefore separated from the adjacent imino groups by a distance considerably

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