mination of drug treatment with 0.5 mg % pyrimethamine. Sonic disruption of cells was accomplished by exposing the cellular suspensions to oscillations of a 9 kc Raytheon oscillator for 20 minutes at 0°C. The cell extracts were then centrifuged for 20 minutes at 2,500 rpm to remove gross flocculent material. The supernatant fluid was further cleared by high speed centrifugation at 15,000 rpm for one hour in a Spinco Model L centrifuge. Antifolic activity was not demonstrated in cell extracts prepared by rapid freezing-and-thawing technics.

Discussion. Ability of a cell to store a drug or active products of the drug *in vitro* suggests an explanation for the prolonged activity of pyrimethamine. The drug or its active product is probably bound to the proteins in the cell and thus prevented from rapid elution from the cell. The failure of conjunctival epithelium to store pyrimethamine must be confirmed; at present there is no obvious explanation for this difference. The demonstration of anti-folic activity in sonic extracts of cells pretreated with pyrimethamine but not in extracts prepared by freezing and thawing is probably an indication of how tightly the drug is bound to the cellular proteins.

Schmidt *et al.*(2) have already shown prolonged activity of pyrimethamine against *Plasmodium cynomolgi* and localization of the drug and or metabolic products indistinguishable therefrom in tissues of monkeys treated with pyrimethamine. The present report shows storage of pyrimethamine at a cellular level in a tissue culture system and defines the conditions necessary to demonstrate such storage.

Summary. 1. Storage of pyrimethamine or active products of pyrimethamine occurs in tissue cultures of monkey kidney treated with the drug. This has been demonstrated by parasite challenges of treated cultures and by microbiological assay with *Streptococcus faecalis R.* 2. The limits of this storage phenomenon have been defined. 3. Some differences in storage of pyrimethamine were noted in monkey kidney cells of various ages. 4. Chick embryo heart explants, HeLa cells and human intestinal cells are able to store pyrimethamine in levels inhibitory to parasite. 5. Mouse mince cultures and KB cells possess a slight ability to store pyrimethamine. 6. Conjunctival epithelium (Chang) shows no evidence of drug storage.

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Reversal of Oxythiamin Toxicity in Neurospora. (23988)

D. V. SIVA SANKAR* (Introduced by Joseph Reilly)
University Biochemical Laboratory, Madras, India

Oxythiamin acts as an antimetabolite of thiamin in the rat and in other organisms(1-5). De Caro *et al.*(5, also 3,4,6) investigated the original finding that oxythiamin increased urinary excretion of thiamin and level of blood pyruvate in the rat, but did not produce the characteristic neuromuscular syndrome produced by administration of pyrithiamin. While both oxythiamin and pyrithiamin could lower the level of thiamin in various tissues, pyrithiamin was much more potent in this respect than oxythiamin. These authors con-

* Present address: Dept. of Chemistry, Adelphi College, Garden City, N. Y.

cluded that pyrithiamin could produce the neuromuscular syndrome since it was a more potent inhibitor of thiamin, whereas oxythiamin could not do so in view of its weaker antagonistic action.

The present paper reports our studies on metabolic effects of oxythiamin in *Neurospora crassa*(8a) (wild strain) and the reversal of these effects by thiamin and by acetate.

Methods. The fungus was grown on the usual medium(8) reported by Horowitz and Beadle. Suitable additions were made before autoclaving, on reversal of oxythiamin toxicity (Table I) while volatile and heat-labile substances were added at room temperature after sterilization by filtering through Seitz filter pad. After growth period of 72 hours, the mycelia were filtered, dried at 105°C to constant weight and the weights recorded. Culture filtrates were analyzed for pyruvic and total ketoacids according to Friedmann and Haugen(9). In experiments on pyruvic decarboxylase activity, the mycelia were dried with acetone and the acetone powders kept refrigerated. Oxidation of pyruvic acid *via* Krebs cycle was minimized by addition of malonate and magnesium chloride. 15 mg of the acetone powder were shaken with 2 mg of pyruvic acid (as sodium salt), 2 mg of potassium malonate and 3 mg of magnesium chloride in total volume of 1.6-1.8 ml in a Warburg flask at 37°C. Carbon dioxide was estimated by the direct method, with and without potassium hydroxide in the center well of the flask.

Growth inhibitory action of oxythiamin on *Neurospora crassa* 8a is completely reversible by either thiamin or by acetate. Succinate and citrate also could reverse this inhibition to a large extent. Whether this might be due to formation of acetate from these 2 substances is hard to decide. A large number of other compounds including acetaldehyde, palmitate and acetoacetate could not reverse toxicity of oxythiamin. This incapacity of acetaldehyde, which is known to be precursor of acetate in the oxidation of pyruvate, shows, though not conclusively, that oxidative decarboxylation of pyruvate involved here might be a single step process bypassing acetaldehyde.

TABLE I. Reversal of Growth Inhibition Produced by Oxythiamin. pH was adjusted to 6.6 before autoclaving. All vessels contained 5 mγ of biotin and, except No. 1 (basal medium), 100 γ of oxythiamin.

Addition		Wt of dried mats, mg
Basal medium without oxythiamin		43.2
Oxythiamin		4.0
Thiamin	(50 γ)	39.3
Acetic acid	(10 mg)	43.0
Citric "	"	22.8
Succinic "	"	33.4
Lactic "	"	3.6
Malic "	"	3.0
Pyruvic "	"	2.9
Oxalic "	"	3.4
Formic "	"	4.1
Propionic "	"	3.0
Ethyl acetoacetate Na.	"	3.0
Formaldehyde	"	3.0
Acetaldehyde	"	3.0
Methanol	"	3.0
Ethanol	"	3.6
Palmitate	"	4.0
$NaHCO_3$	"	3.6
$(NH_4)_2HPO_4$		4.0

However, formation of acetaldehyde by *Neurospora* has been shown by Strauss(7). More recent work(11) shows that the metabolic function of lipoic acid includes oxidative decarboxylation of pyruvate. However, while bicarbonate could replace the pyruvate oxidation factor in *Streptococcus faecalis*(10), it could not reverse oxythiamin toxicity in our experiments. Further, while acetate helps growth of *Neurospora* in the presence of oxythiamin, it does not help accumulation of keto and pyruvic acids. This may be attributed to inhibition of decarboxylation of pyruvate by oxythiamin. The larger amounts of acetate added seem to prevent further conversion of pyruvate to acetate, thus increasing the accumulation of pyruvic acid. This is plausible on grounds of mass action law. Citrate and succinate, which do not help growth of oxythiamin-inhibited fungus as much, do not lead to as high accumulation of ketoacids. Neither do they, on the other hand, decrease their content.

Inability of palmitate to reverse toxicity of oxythiamin, by giving rise to acetate, seems to support Lein's conclusion(12) that palmitate and stearate are inert in *Neurospora* in relation to formation of acetate. Strauss(7)

TABLE II. Metabolic Effects of Oxythiamin, and Their Reversal. 10 ml medium, 72 hr growth, pH 6.6.

Addition to basal medium	Mat wt, mg	Total keto-acids, mg	Pyruvic acid, mg
Oxythiamin (100 γ)	3.0	5.80	5.50
Oxythiamin + thiamin (50 γ)	33.6	4.0	.8
Oxythiamin + acetic acid (10 mg)	37.9	26.8	21.2
Oxythiamin + citric " (10 mg)	27.4	4.25	.85
Oxythiamin + succinic " (10 mg)	28.1	6.55	6.55

indicates 2 routes for formation of acetate in oxidative decarboxylation of pyruvate, one mediated by pyruvic decarboxylase, and the other not. Strauss(7) has also shown low carboxylase activity in some strains of *Neurospora crassa*.

Accumulation of ketoacids, and the higher percentage of pyruvic acid in total ketoacids in media with oxythiamin is interesting. These results (Table II) denote a similarity of function of thiamin in several organisms, including the rice moth larva (*Corcyra cephalonica St.*(13) and the rat(1). Table III shows the effect of incorporation of oxythiamin, and of both thiamin and oxythiamin, in culture media, on decarboxylation of pyruvate by mycelial preparations. Addition of oxythiamin to basal medium might be considered as equivalent to *in vivo* deficiency of thiamin, while addition of antivitamin to a mycelial preparation obtained from normal medium might be viewed as *in vitro* deficiency. While growth was depressed to a large extent by *in vivo* addition of oxythiamin, pyruvic decarboxylase activity of the small amount of mycelium obtained, was not strikingly decreased. On the other hand, *in vitro* addition of antivitamin abolished enzymatic activity. This inhibition was completely counteracted by thiamin and not by acetate (the results with acetate are not shown in the present communication, since they are negative).

TABLE III. *In Vitro* and *In Vivo* Effect of Oxythiamin on Pyruvic Decarboxylase Activity of *N. crassa* Mycelial Acetone Powders.

No.	Detail	μl CO_2 evolved per mg tissue per hr
1.	*N. crassa* grown in medium containing 100 γ of oxythiamin/10 ml	7.81
2.	*Idem* and 50 γ thiamin/10 ml	15.5
3.	Acetone powder from No. 1 with 50 γ thiamin added *in vitro*	12.3
4.	Mycelium grown in basal medium	9.64
5.	Acetone powder from No. 4 with 100 γ oxythiamin added *in vitro*	.0
6.	*Idem* + 50 γ thiamin	11.27

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