exposed (till they lost equilibrium) to 38·3° C and brought back to acclimation temperature of 22·5° C, initial recovery was cent per cent but subsequently 38% mortality occurred in 24 hr, 50% mortality in 72 hr, and 75% mortality occurred in 96 hr (Fig. 1). It appears, therefore, that thermal shock had impaired the capacity of fry to survive for a prolonged period at the ambient water temperature.

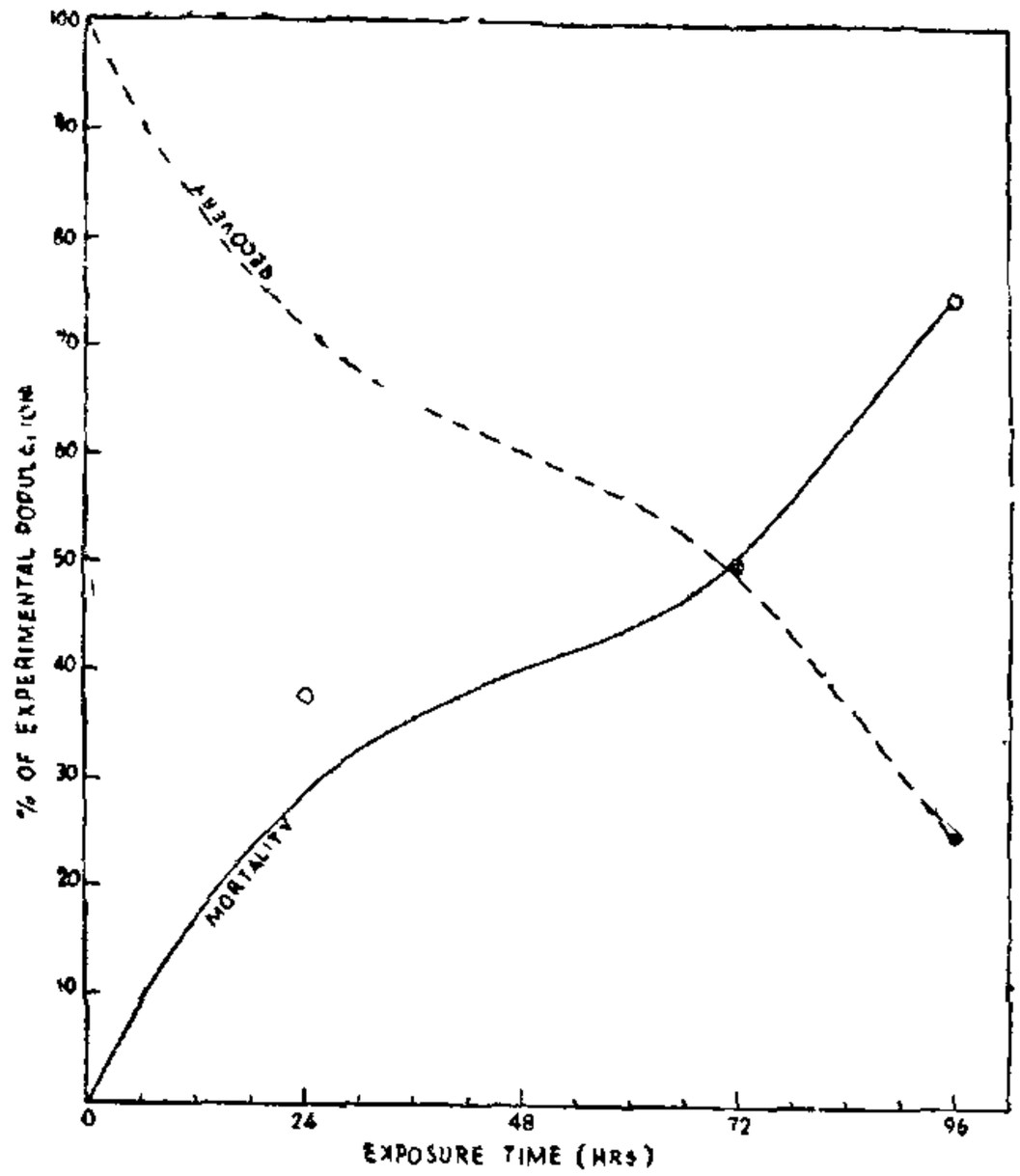

FIG. 1. Recovery and latent mortality of *Catla catla* fry at 22·5° C after exposure to thermal shock at 38·3° C ± ·1.

In the second set of experiments prolonged effect of exposure to 38·4° C was observed. Mortality seen in the fry at 22·5° C (acclimated water temperature) after 6 and 8 minutes' exposure to 38·4° C are markedly higher, *i.e.*, 17% and 33% in 48 hr, compared to the nil mortality when the exposure was restricted to 4 minutes or less (Table II).

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STUDIES ON ACETYLATION OF SULFANILAMIDE IN *OCHROMONAS MALHAMENSIS*

ABSTRACT

O. malhamensis metabolises sulfanilamide to a non-diazotisable derivative, N-acetyle sulfanilamide. Vitamin B_{12} and methionine stimulate acetylation of sulfanilamide *in vivo*. The acetylation of sulfanilamide could be demonstrated *in vitro*. The para amino benzoic acid and sulfanilamide acetylating systems are inhibited by the presence of the other in the growth medium either way.

ACETYLATION of sulfanilamide, discovered by Marshall *et al.*[1] has been the subject of a large number of investigations. Since a considerable fraction of the drug may circulate and be excreted in the acetylated form, the phenomenon has practical importance[2–4]. In *Ochromonas malhamensis* sulphanilamide growth inhibition has been observed by Potty and Tamhane[5]. Whether a disposal system for sulfanilamide by way of acetylation exists in this organism has been the subject-matter of this report. A *p*ABA acetylation mechanism has been studied earlier[6].

EXPERIMENTAL

Maintenance.—*O. malhamensis* was maintained by weekly transfers in Fords medium[7] and incubated under light at 28° C for 7 days.

Growth Studies.—These were conducted in the basal medium of Johnson *et al.*[8], in pyrex test-tube in a final volume of 4 ml. 2 drops of thrice diluted, 5-day old culture, was used as the inoculum. The growth was measured after diluting to a final volume of 10 ml in a Klett-Summerson photoelectric colorimeter at 660 mμ.

Cultivation of O. malhamensis *for* in vitro *studies.*—The cells were grown in 100 ml batches in 500 ml Erlenmeyer flasks, recovered by centrifugation under refrigeration, washed twice with chilled distilled water and suspended in a suitable volume of water.

Estimation of sulfanilamide.—The method of Bratton and Marshall[9] involving diazotisation and subsequent coupling with N-(1-naphthyl) ethylene diamine dihydrochloride and measuring the colour intensity at 540 mμ was followed for the estimation of sulfanilamide. Optimum conditions for the hydrolysis involved the boiling of samples with 4 N HCl for one hour in a water-bath.

In vitro *studies on the acetylation of sulfanilamide.*— Cell suspensions corresponding to 80 mgm were added to a reaction system consisting of 0·04 M phosphate buffer, pH 7·0, and ·7 μ moles of sulfanilamide and other additions where stated in a final volume of 10 ml. This was incubated at 30° C under constant shaking for 20 hours after which

the reaction was stopped by additions of trichloroacetic acid to a final concentration of 10%.

Results and Discussion

O. malhamensis has been shown to be carrying an acetyl tranferase which inactivates the toxicity of *p*-aminobenzoic acid through acetylation. The supposition is based on the observation that unlike *p*ABA, N-acetyl *p*ABA is not toxic to the organism over very large ranges of concentrations. Sulfanilamide was found to be toxic to the organism[5] and as the role of the acetylating enzymes in the mechanism of drug resistance is significant[10], it seemed interesting to study the activity of this enzyme in *O. malhamensis*.

The presence of sulfanilamide derivative, not reacting with Bratton-Marshall reagent but showing reaction only on hydrolysis with acid in the culture filtrate was observed during the course of the present studies (Table I) at sub-inhibitory levels of sulfanilamide pointing to the operation of a detoxification mechanism involving the amino group of sulfanilamide.

Table I

In vivo *acetylation of sulfanilamide in* O. malhamensis

Amount of sulfanilamide* added to growth medium μg/ml	Inhibition %	Amount of sulfanilamide present in the medium after 7 days		Amount converted to acetylsulfanilamide %
		Before hydrolysis μg/ml	After hydrolysis μg/ml	
0	..	..	..	..
50	20·2	15·0	46·0	62
100	40·2	41·0	96·0	55
150	60·2	71·5	139·0	45
200	80·5	140·0	188·0	24
250	99·2	203·0	225·5	9

* Supplement included in a final volume of 4 ml of the medium of Johnson *et al.*[8].

The acetylation of sulfanilamide was inferred from the stimulation of the production of this compound by acetate and substances contributing to the formation of acetyl COA, *viz.*, pantothenate and aspartic acid as shown in Tables III and IV and the analogy of similar phenomenon with *p*ABA where N-acetyl PABA was indentified as the metabolite[5]. The acetylation of sulfanilamide seems to be correlated to the growth of the organism as shown in Table I. At lower level when the growth is sufficiently good the acetylation of sulfanilamide is also more. At high substrate concentrations not only is the growth suppressed but the activity of the acetylating enzyme is also arrested.

O. malhamensis has been shown to possess the capacity of acetylating *p*-ABA[6] as well as sulfanilamide. However, when the effect of exogenous addition of one in the growth medium, on the *in vitro* acetylation of the other was tried, it was found that either way there was no acetylation.

This could point to the fact that the enzymes were specific for the two substrates, one being rendered ineffective, when grown even at subinhibitory levels of the other (50 μg for *p*ABA, 125 μg for sulfanilmide).

The effect of various metabolites on the *in vivo* acetylation was tried; of these only vitamin B_{12} and methionine (Table II) were found to enhance the enzyme activity at 50% inhibition level to some extent. This could be due to the prominent role of B_{12} in the metabolism of the organism in view of its absolute requirement for growth and its capability to reverse the growth inhibition of sulfanilamide at 50% inhibitory level[5].

Table II

Effect of some metabolites on the in vivo *acetylation of sulfanilamide*

Supplements to the growth medium*	Concentration ug/ml	% acetylation of sulfanilamide	
		Sulfanilamide added in ug/ml	
		125	250
None ..	..	42·8	8·2
Vitamin B_{12} ..	1·0	51·4	10·0
Methionine ..	250·0	51·4	10·4

* Supplement included in a final volume of 4 ml of the medium of Johnson *et al.*[8].

Table III

Effect of various metabolites on acetylation of sulfanilamide in vitro *by* O. malhamensis

Supplement added to the system	Concentration of addition in μg	Acetyl sulfanilamide* formed per mg dry weight of cells
None ..	..	1·75
Vitamin B_{12} ..	1·0	1·8
dl-Methionine ..	1000	1·75
Calcium pantothenate	1000	2·20
Pyridoxine ..	1000	2·25
Biotin ..	1000	2·0
Sodium acetate ..	1000	2·0
Adenine ..	1000	1·85

* Acetyl sulfanilamide expressed as equivalent to μg of sulfanilamide obtained after acid hydrolysis.

TABLE IV

Effect of various metabolites added to the growth medium on the acetylation of sulfanilamide in vitro

Supplements added to growth medium	Concentration µg/ml	Acetyl sulfanilamide formed per mg dry weight of cells	Acetyl PABA* formed per mg dry weight of cell
1. None		1·698	5·25
2. *p*ABA ..	50	..	4·90
3. Sulfanilamide ..	125	1·483	..
4. 3 + Calcium pantothenate	10	1·74	..
5. 4 + *dl*-aspartic acid	25	1·98	..
6. 5 + Sodium acetate ..	50	2·36	..

* *In vitro* reaction mixture for *p*ABA contained 7 µ moles of *p*ABA instead of sulfanilamide. Acetyl *p*ABA was computed from the difference in the arylamine before and after hydrolysis.

Methionine has been shown to spare to some extent, the requirement of vitamin B_{12} in this organism. Also methionine has been shown to increase the calcium pantothenate levels in *E. coli* and *B. subtilis* grown independent of vitamin B_{12}[11].

The demonstration of *in vitro* acetylating system in *O. malhamensis* was established with respiring cells (Tables III and IV). An interesting point of the present study is the rather long period required for the maximum conversion of sulfanilamide. Probably permeability factors are involved in this. Similar results were observed in the case of *p*ABA acetylating enzyme[6] in this organism.

Aerobic conditions favoured the synthesis of acetyl sulfanilamide *in vitro* as provided by incubating the system in rotary shakers.

With pigeon liver extracts the similar results have been observed[12,13]. The present studies demonstrate the development of sulfa-acetylating system in *O. malhamensis* in presence of sub-inhibitory levels of the drug.

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ANALYSIS OF METHACROLEIN IN GAS MIXTURES

METHACROLEIN, an unsaturated aldehyde, can be estimated by several methods[1] applicable to carbonyl compounds, such as oxime method, hydrazone method, bisulphite method, dimedone method[2] and polarographic method[3]. However, these have been developed for analysis of carbonyl compounds only in solution. A method is described here for estimating methacrolein in gas mixtures.

Methacrolein is absorbed quantitatively in concentrated sulphuric acid and so can be estimated in this way. The method cannot be used, however, if the methacrolein vapour is mixed with gaseous olefins. The following method has been successfully employed for analysing methacrolein in product gas mixtures that result from air oxidation of isobutene over bismuth molybdate or ferric molybdate catalyst[4] at 350°–500° C, and consist of isobutene (I), methacrolein (II), carbon monoxide (III), carbon dioxide (IV), oxygen (V), and nitrogen (VI), a typical composition being I 5·0%, II 1·5%, III 1·0%, IV 1·0%, V 16·0% and VI 75·5%. An orsat-type gas analysis set-up is employed. For absorbing methacrolein a saturated solution of dimedone (5 : 5-dimethyl cyclohexane-1 : 3-dione) in an aqueous solution of sodium sulphate (12%) is used.

While dimedone is the active reagent forming an insoluble product with II, sodium sulphate is added only to reduce the solubility of other gases in the solution. It is however found that though the reagent does not absorb I, III and V, it does absorb IV to some extent. In view of this, analysis is done twice taking two samples of the same gas mixture. In one case, the total volume (V_1) of II and IV in the gas mixture is measured by absorption in dimedone solution followed by KOH solution, and I, V and III are then estimated, in this