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Of

Haffkine Institute



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Parel, Bombay - 400 012, India

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Bulletin of Haffkine Institute

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In vitro synergistic antibacterial activity of trimethoprim and furazolidone

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Abstract

MICs for trimethoprim and furazolidone, both individually and in combination were determined against 50 isolates of gram negative bacilli. MIC values ranged from 0.25 mcg/ml to 32 mcg/ml for trimethoprim and 6.25 mcg/ml to 200 mcg/ml for furazolidone. Synergistic activity for trimethoprim and furazolidone was seen in 39 out of 50 isolates. In eight isolates the combination was found to have additive effect and only in three isolates neither synergistic nor additive effect could be seen for the combination.

Introduction :

A combination of trimethoprim and sulphamethoxazole was considered as one of the best examples of synergistic antibacterial activity at the time of its introduction. It was also thought that sulphamethoxazole protects trimethoprim from the acquisition of resistance (1). However, the validity of the latter is highly questionable since majority of hospital gram negative bacterial isolates have been noted to be resistant to the sulphonamides (1). Moreover resistance to the combination has been increasingly reported in the recent years (2). On the other hand nitrofurans derivatives still enjoy a reputation of high order of susceptibility towards gram negative bacilli. Resistance of gram negative bacilli for nitrofurans mediated through R-plasmids has not been reported so far and the development of chromosomal resistance may be considered a rare

event (3). However, its side effects such as nausea and vomiting raise major problems in the administration of its full therapeutic dose (3). Recently, both clinical and in vitro studies have shown a possibility of reduction of therapeutic dose for nitrofurantoin by the use of combination of trimethoprim and nitrofurantoin in urinary tract infections (UTI) (4). Furazolidone, another nitro-furan derivative has been successfully used to reduce the quantum of fluids to be transfused in cholera cases (5). It has also been used effectively in case of shigella dysentery (6). In view of this, the present work was carried out to study the in vitro effect of trimethoprim and furazolidone combination on pathogenic isolates.

Materials and Methods

Bacterial isolates :

Totally 50 bacterial isolates from pathological samples in various hospitals in

Bombay were employed in the present study. The actual number of isolates of each species employed is shown in Table I. Enterotoxicity of enterotoxigenic *Escherichia coli* (E T E C) was tested by adult rabbit ileal loop technique (7).

Drug susceptibility testing :

Diluent free trimethoprim and furazolidone were kindly supplied by M/s. Uni-chem Laboratories. The drugs were dissolved in (conc.) Lactic acid and 1N NaOH respectively. For determination of minimum inhibitory concentration (MIC) for individual drugs and for combination, agar dilution method (8) was used. Drug sensitivity agar (Oxoid formula) was used through out the study.

Fractional inhibitory concentration :

(FIC) was calculated as the ratio of MIC of the drug in combination to MIC of the drug alone.

FIC Index :

The sum of FICs of trimethoprim and furazolidone for each organism gave the FIC index (9). FIC index less than one was considered as the indication of synergy and 1.0 as indication of additive effect.

Results :

Range of MIC values for trimethoprim and furazolidone (individually) are shown in Table I. Wide range of susceptibilities can be seen for enterotoxigenic *E. coli*. MIC values for trimethoprim ranged from 0.25 mcg/ml to 32 mcg/ml while for furazolidone from 6.25 mcg/ml to 200 mcg/ml. In case of *Salmonella* species majority of the strains were found to be less sensitive to both, trimetho-

prim (MIC 8-32 mcg/ml) and furazolidone (MIC 25-200 mcg/ml).

FIC indices for the combination of trimethoprim and furazolidone are shown in Table II. FIC index less than one, implying the synergistic activity was observed in 39 out of 50 isolates. Additive effect of the drugs in combination was seen in 8 isolates. Neither synergistic nor additive effect could be seen for two *V. cholerae* and one *E. coli* strains isolated.

Discussion :

MICs for trimethoprim and furazolidone, both individually and in combination were determined against 50 isolates in the present study. Except for one *E. coli* and seven *Salmonella* strains all the isolates were sensitive to less than 50 mcg/ml of furazolidone. The 8 isolates which were less sensitive to furazolidone were however, found to be sensitive at 100-200 mcg/ml, indicating that none of them were totally resistant to furazolidone. Similarly, majority of the isolates (35/50) were sensitive to trimethoprim at a concentration of less than 4 mcg/ml and all were sensitive at or below 32 mcg/ml. This is an interesting observation in the light of recent report from our laboratory which indicated a sulphonamide resistance to the tune of 90 per cent among large number of isolates (10). The present situation is analogous to the reports from UK and many other countries where proportion of strains sensitive to trimethoprim has remained high despite heavy use of combination of sulphanamide and trimethoprim (1). More and more reports are currently being published where trimethoprim was

Table I
M I C values for individual drug against gram negative bacilli.

Organisms	No. of isolates	M I C Trimethoprim (mcg/ml)				M I C Furazolidone (mcg/ml)			
		0.25	0.5	2.0-4.0	8.0-32.0	6.25	12.5	25-50	100-200
E T E C	27	9	7	6	5	13	9	4	1
<i>Salmonella typhimurium</i>	12	—	1	1	10	—	—	5	7
<i>Salmonella enteritidis</i>	1	1	—	—	—	—	1	—	—
<i>Shigella flexneri</i>	2	2	—	—	—	1	—	1	—
<i>Shigella Shiga</i>	1	1	—	—	—	1	—	—	—
<i>Vibrio cholerae</i>	6	—	2	4	—	6	—	—	—
<i>Vibrio parahaemolyticus</i>	1	—	—	1	—	—	1	—	—

Table II
Effect of trimethoprim and furazolidone combination on gram negative bacilli

Organisms	No. Tested	F I C Index						No. showing Syner-gistic Effect	No. showing Additive Effect	No. showing no Syner-gistic Additive Effect
		0.5	0.5	0.5-0.7	0.7-0.9	1	> 1			
<i>E. coli</i>	27	—	4	3	19	—	1	26	—	1
<i>Salmonella sp.</i>	13	1	—	—	4	8	—	5	8	—
<i>Shigella sp.</i>	3	—	—	1	2	—	—	3	—	—
<i>V. cholerae</i>	6	—	4	—	—	—	2	4	—	2
<i>V. parahaemolyticus</i>	1	—	—	—	1	—	—	1	—	—

found to be considerably more effective than sulpha against most of the bacterial species (1). In UTI isolates, the activity of trimethoprim is so dominant as to be almost entirely responsible for the activity of co-trimoxazole and this is the reason for consideration of trimethoprim as a single drug in UTI in Britain. But a fear of development of trimethoprim resistance is expressed for the use of trimethoprim as a single drug (1). In view of this a combination of trimethoprim and furazolidone may offer the following advantages. Resistance for trimethoprim does not appear to be common and chances of development of resistance towards furazolidone may be quite rare. Further, a possibility of R-plasmid mediated resistance for the combination of trimethoprim and furazolidone, as in case of co-trimoxazole, is unlikely. Synergistic activity of the combination may help to reduce the therapeutic dose

of furazolidone and may avoid its side reactions. Hence the synergism observed in the present study on fifty different isolates is very significant.

The need of antimicrobial chemotherapy is more pronounced in case of enteropathogens which cause systemic form of the diseases. In case of *S. typhi*, R-plasmid mediated chloramphenicol resistance, although not common, has caused a grave threat. However such a possibility seems to be unlikely for furazolidone. We hope that a combination of trimethoprim and furazolidone may prove an effective chemotherapy for enteric fever since both furazolidone (11) and trimethoprim (12) have been successfully used in the treatment of typhoid. In vitro drug susceptibility testing of trimethoprim and furazolidone, both individually and in combination against enteric fever bacilli is currently in progress in our laboratory.

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Studies in Anaphylaxis I. A model of anaphylactoid oedema for screening antianaphylactic agents

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Compound 48/80, a polymeric agent, produces reactions, which simulate anaphylaxis, known as anaphylactoid reactions. Local injections of the compound in the hind paw of mice induce an oedema, which is measured as function of change in paw thickness. The induction of oedema is rapid in onset, and the reaction subsides within 60 min.

The oedematous response of the animal bears a linear relation to the dose of the compound injected, over a short dose range. Studies using specific blocking agents suggest an endogenous release of histamine to be involved in the genesis of the oedema, 5-hydroxytryptamine does not appear to be involved in the oedema formation. The oedema formation is further sensitive to changes in calcium ion concentrations and requires metabolic energy.

Introduction :

'Anaphylactoid reactions' is a term coined by Selye (1937) to represent reactions which are similar to anaphylactic reactions, but do not involve an interaction of antigens and antibodies. Most mediators of anaphylaxis and a variety of substances like dextran, egg white, yeast, hyaluronidase, polyvinylpyrrolidone have been demonstrated to induce such reactions (Selye 1937, Elster *et al* 1949, van Arman *et al* 1965, Parrot and West 1957, Halpern and Briot 1952).

Compound 48/80, a polymeric agent obtained by the condensation of p-methyl-phenylethyl-methylamine with formaldehyde (Paton 1951), is one of the most potent liberators of histamine known. In addition to releasing histamine it has been demonstrated to release

5-hydroxytryptamine and Slow Reacting Substance of Anaphylaxis (SRS-A) (Halpern *et al* 1957, Chakravorty *et al* 1960, Chasin *et al* 1980).

Increasing interest in compound 48/80 induced anaphylactoid reactions, has been generated by the demonstration that 48/80 induced lethality could form a basis for the study of inhibitors of mast cell mediated shock (Niemegeers *et al* 1978). Chasin *et al* (1979) demonstrated that 48/80 could release radiolabelled 5-hydroxytryptamine from preloaded mast cells, and that inhibition of this release could be equated with antianaphylactic potency. While both the above mentioned tests are expensive to perform, they are without doubt simpler than the existing models for antianaphylactic screening. We have standardised a simple model of anaphylactoid nature using compound

48/80, to be finally used for antianaphylactic screening. The present communication describes the basic characteristics of the model.

Materials and Methods

Compound 48/80 was obtained from the Sigma Chemical Co., U.S.A., the compound was dissolved in distilled water to obtain a concentration of 100 mcg/ml, which was stored at -20°C in aliquots of 1 ml each. No solution of the compound was re-refrigerated or reused.

All other chemicals and drugs used were of the standard purity and grade.

Paw thickness measurements were made with a pocket thickness gauge of the Mitutoyo Manufacturing Co., Japan. As the instrument was calibrated in inches, no attempt was made to convert the values in metric measures, but the results were expressed in units, each unit being equal to 0.001 inch.

Mice of either sex, weighing between 18 and 22 g. were obtained from Haffkine Bio-Pharmaceuticals Corporation Ltd., Animals of the Kasauli and the N.I.H. Strain were used for the study. Prior to the administration of the compound 48/80, the hind paw thickness of both paws was measured. Next the compound in appropriate dose dissolved in 0.05 ml was injected intrapedally (ipd.) in the

right paw, the left paw received 0.05 ml saline. The increase in paw thickness was calculated after measuring the thickness 5, 10, 20 or more min. after the administration of the compound. Difference in the increase in thickness in the right and the left paws was the oedema induced by 48/80. Statistical significance of the data obtained was calculated using Student's 't' test.

To study the influence of standard agents, on the oedema formation, they were administered intraperitoneally (ip) 30 min. prior to 48/80 or locally along with 48/80.

Results

1. Time course of oedema formation: Compound 48/80 (0.3 mcg) caused the induction of oedema which was measurable at 5 min. after the administration of the compound. The peak oedema formation was at 10 min. and the oedema subsided to low level at 60 min. Table I shows the time course of the oedema in response to 0.3 mcg. of 48/80, 0.2 mcg. of histamine and 0.2 mcg. of 5-hydroxytryptamine.

2. Dose response relationship for 48/80: The compound in doses ranging from 0.078 to 0.312 mcg elicited a dose dependent oedema in mice. Higher doses (upto 5.0 mcg.) produced a res-

Table I
Time course of mouse hind paw oedema (in units)

Time	Compd. 48/80	Histamine	5 HT
5 min.	12.33(1.08)	13.16(0.87)	14.15(1.22)
10 min.	22.83(0.56)	21.66(0.62)	23.00(0.96)
30 min.	12.00(1.06)	9.83(1.08)	12.66(1.14)
60 min.	4.00(1.34)	1.33(1.26)	2.00(0.56)

Figures in parenthesis indicate SE of mean, $n = 6$.

ponse which was 50% of the maximal response. Fig. 1 shows the relation of the dose to the oedema formed.

3. Variation of response on the basis of sex: Using 50 male and 50 female mice of the Kasauli strain no difference in the response to 0.3 mcg. of 48/80 was observed. Table II shows the results.

4. Variation of response on the basis of strain: As shown in Table III, the response of N.I.H. strain was higher than that of Kasauli strain, when equal amounts of 48/80 were injected.

5. Tachyphylaxis to 48/80: Repeated daily doses of compound 48/80 produced a rapid tachyphylaxis of the response. Histamine in a similar dose schedule showed no such phenomenon. Table IV shows the reduction in response to 48/80, and the response to histamine on daily administration.

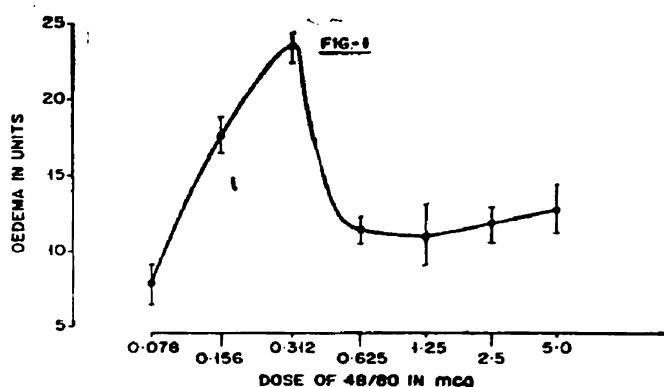


Table III

Variation of response to 0.3 mcg. 48/80 on the basis of strain

Units of oedema	% of total animals giving the response	
	N.I.H.	Kasauli
12 — 15	—	9.0
16 — 19	—	16.2
20 — 23	8.0	42.4
24 — 27	16.0	24.3
28 — 31	40.0	8.1
32 — 35	32.0	—
36 — 39	8.0	—

Table II

Sex variation in response to 48/80

Sex	Oedema	S. E.
Males	22.64	0.32
Females	22.42	0.40

Not significant

P < 0.1

Table IV

Effects of repeated daily doses of 48/80 and histamine

	Histamine		48/80	
	Oedema units	S. E.	Oedema units	S. E.
Day 1.	21.66	0.62	22.83	0.56
Day 2.	20.50*	1.08	10.66**	1.33
Day 3.	22.00*	1.22	4.33**	0.96

number of animals — 6, dose of 48/80- 0.3 mcg.
dose of histamine 0.2 mcg.

*Not significant. **P < 0.01

6. Effect of antihistaminic and antiserotonin agents: Mepyramine maleate (1 mg/kg) caused a 43.8% inhibition of the oedema produced, while methysergide bimalate (1 mg/kg) failed to influence the 48/80 induced oedema.

7. Effect of ethylenediaminetetraacetic acid and sodium fluoride: EDTA at concentrations 1 and 2 mM caused dose related inhibition of oedema formation. Sodium fluoride also inhibited the oedema formation in a dose dependant fashion. Table V shows the results.

Table V

Effect of EDTA and NaF on compound 48/80 induced oedema

Treatment	Oedema	S. E.
—	20.00	0.62
EDTA 1 mM	12.00*	0.00
EDTA 2 mM	6.5*	0.92
NaF		
0.25 mM	16.50*	0.76
0.50 mM	14.60*	1.02
1.00 mM	10.33*	0.56

n = 6. *p > 0.01

Discussion

Compound 48/80, histamine and 5-hydroxytryptamine all induce a rapidly developing oedema on intrapedal administration. The time course for the formation of oedema, being similar to that for histamine and 5-hydroxytryptamine, suggests that these two amines could be involved in its formation. The rapid onset and short duration of the response appears to rule out the involvement of the Slow Reacting Substance of Anaphylaxis in the model studied.

Compound 48/80 produced a dose response curve which is both rare and without explanation at this juncture. The response of higher doses of the compound seems to be inhibited by a secondary effect on some antagonistic receptor. It is also possible that compound 48/80 inhibits its own action at higher doses.

Absence of variation of the response on the basis of sex was expected, the difference in response of the two strains of animals used could be due to their inherent differences in reactivity.

Repeated daily doses of 48/80 caused a tachyphylactic response which was not observed with histamine. The induction of tachyphylaxis is probably indicative of an indirect effect of 48/80 in producing an oedema. A depletion of endogenous amines probably takes place, producing the tachyphylaxis.

Mepyramine maleate produced a significant inhibition of the 48/80 induced oedema suggesting the involvement of histamine. The absence of any influence of methysergide on oedema formation rules out the mediation of 5-hydroxytryptamine in the response, which is in contradiction to the observations of Chasin *et al* (1979).

The release of mediators by antigens, in sensitised animals is both energy and calcium dependant. We have for this reason studied the effects of EDTA and NaF on the 48/80 induced oedema. The inhibitory effects of both go to suggest a similarity between this reaction and the true anaphylactic reactions studied by Mongar and Schild (1958) and Chakravorty (1959).

Compound 48/80 induced mouse hind paw oedema is a simple test which shows some similarity to anaphylactic reactions and may find use as a preliminary screening test for inhibitors of mast cell mediated shock.

Acknowledgements

The authors are grateful to Dr. K. D. Sharma, Director, Haffkine Institute, for the facilities provided for the study.

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Effects of maternal protein deficiency on brain maturation of the rat progeny

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The comparative effects of maternal protein deficiency (5% protein) during gestation and/or lactation period(s) on the rat progeny were assessed by evaluating brain DNA and protein content from birth to weaning. Protein deficiency was imposed either on day of conception or parturition. The maternal protein deficiency imposed during both periods had comparatively more adverse effect on brain cell number (DNA content), of the progeny, as compared to protein deficiency during lactation period alone. However, cell size (protein/DNA ratio) was not altered in both the deficient groups.

Introduction :

Several observations suggest that morphological and biochemical alterations occur in the central nervous system of the experimentally undernourished animals (1).

Growth of a cell can be assessed by various biochemical parameters. The serial values of DNA, an estimate of cell number, and of protein, an estimate of cell size, at different ages suggest that growth in the rat brain from birth until 13th day occurs by hyperplasia, from 13th day to 17th day by both hyperplasia and hypertrophy and after 17th day by hypertrophy alone (2-4).

Winick and Noble (4, 5) reported significant deficits in whole brain DNA and protein content in the rats undernourished during postnatal period by litter size manipulation. Similar deficits in whole brain DNA values were associated with

postnatal undernutrition brought about by maternal protein deficiency combined with litter size changes (6). Maternal protein deficiency during gestation and lactation also lowered brain DNA and protein levels (7). However there is no report on the comparative effects of maternal protein deficiency during gestation and/or lactation period(s) on these two parameters in the rat progeny at different stages of brain maturation. The work presented here concerns to such comparative effects.

Materials and Methods :

Adult female albino rats of proved fertility weighing 180-200 g (bred at Haffkine Institute) were used for the experiments. They were divided into three groups, each receiving a different dietary regimen from the first day of conception (sperm positive dams) or parturition. The first group (G^+L^+) was fed 20 per cent protein diet during gesta-

Table 1

Composition of Diets (g per kg diet).		
	Control	Experimental
Casein	240	60
Vitamin mixture ¹	20	20
Salt mixture ²	40	40
Ground nut oil	70	70
Sucrose	200	200
Potato starch	430	610

(1) Based on NAS-NRC recommendation (13) and fat soluble vitamins were separately added at the following levels (per kg diet): Vitamin A, 4.0 mg, Vitamin D, 31.25 mg, Vitamin E, 300.0 mg, and Vitamin K, 5.0 mg as detailed in reference 14.

(2) Hawk-Oser salt mixture no. 4 as described in reference 15.

Table 2

Effect of maternal protein deficiency during gestation and/or lactation on the body weight and brain weight of the progeny at different ages.¹

Age (days)	Group		Body Wt. (g)		Brain Wt. (g)		Brain Wt. Body Wt.	
			Mean	S.E.	Mean	S.E.	Mean	S.E.
At Birth	G ⁺	(13) ²	6.10	0.14	0.269	.004	.044	.001
	G ⁻	(26)	4.4	0.06*** ³	0.201	.017**	.047	.001*
7	G ⁺ L ⁺	(8) ¹	17.0	0.7	0.734	.014	.042	.001
	G ⁺ L ⁻	(8)	10.0	0.6 **	0.655	.012*	.068	.003*
	G ⁻ L ⁻	(8)	7.0	0.5 **	0.513	.010**	.071	.003*
14	G ⁺ L ⁺	(7)	27.0	0.4	1.204	.034	.044	.001
	G ⁺ L ⁻	(8)	11.0	0.8 **	0.898	.054**	.082	.001*
	G ⁻ L ⁻	(8)	8.0	0.4 **	0.704	.020**	.087	.001*
21	G ⁺ L ⁺	(6)	44.0	0.9	1.380	.014	.032	.001
	G ⁺ L ⁻	(7)	13.0	0.7 *	1.111	.030**	.086	.003**
	G ⁻ L ⁻	(7)	12.0	0.5 *	1.040	.033**	.086	.004**

(1) Dams were fed on 20 per cent (High Protein, HP) or 5 per cent (Low Protein, LP) protein diet from first day of conception or delivery till weaning. G⁺ L⁺, HP diet during gestation and lactation periods; G⁺ L⁻, HP diet during gestation and LP during lactation, G⁻ L⁻, LP diet during gestation and lactation periods.

(2) Numbers in parenthesis indicate number of observations.

(3) Values significantly different from G⁺ L⁺ group

* P < .05; **P < .01;

Table 3

Effects of maternal protein deficiency during gestation and/or lactation on brain protein and DNA of the progeny at different ages.¹

Age (days)	Group		Protein (mg)			Brain DNA (mg)			Protein/DNA	
			Mean	S.E.		Mean	S.E.		Mean	S.E.
At	G ⁺	(13) ²	15.05	1.05		0.515	0.046		29.29	0.22
	G ⁻	(26)	11.22	0.24** ⁴		0.382	0.008*		29.40	0.23
7	G ⁺ L ⁺	(8)	42.65	1.33	(183) ³	1.490	0.084	(189)	28.80	0.74
	G ⁺ L ⁻	(8)	37.72	1.11*	(150)	1.250	0.062*	(143)	30.40	0.99
	G ⁻ L ⁻	(8)	29.70	0.93	(165)	0.971	0.031**	(154)	30.70	0.56
14	G ⁺ L ⁺	(7)	91.30	2.24	(114)	2.62	0.14	(76)	35.43	2.04
	G ⁺ L ⁻	(8)	67.44	5.13**	(79)	1.84	0.13 *	(47)	36.23	0.77
	G ⁻ L ⁻	(8)	51.08	2.88**	(72)	1.41	0.06 **	(45)	36.07	0.97
21	G ⁺ L ⁺	(6)	144.36	8.98	(58)	3.15	0.17	(20)	48.91	4.95
	G ⁺ L ⁻	(7)	105.74	3.82*	(57)	2.36	0.07 *	(28)	44.69	1.05
	G ⁻ L ⁻	(7)	98.52	3.64**	(93)	2.19	0.09 **	(55)	45.02	0.74

(1) Dams were fed on 20% (High Protein, HP) or 5% (Low Protein, LP) protein diet from first day of conception or delivery till weaning. G⁺ L⁺ HP diet during gestation and lactation periods; G⁺ L⁻ HP diet during gestation and LP during lactation, G⁻ L⁻ LP diet during gestation and lactation periods.

(2) Numbers in parenthesis indicate number of observations.

(3) Values in adjacent parenthesis indicate weekly per cent increment in respective groups.

(4) Values significantly different from G⁺ L⁺ group *p < .05, **p < .01.

tion and lactation and this served as a control. The second group (G^+L^-) was fed 20 per cent protein diet during gestation and 5 per cent protein diet during lactation. The third group (G^-L^-) was fed 5 per cent protein diet during gestation and lactation. The water and the diet were provided *ad libitum* to all the groups. The composition of the diet is presented in Table 1.

Pups were killed by decapitation at birth, 7, 14 and 21 days of age. Brain was removed alongwith the olfactory lobes and processed further as reported earlier (8). DNA was estimated by the method of Schneider (9) and determined by the diphenyl amine reaction (10). Protein was estimated by the method of Lowry *et al* (11).

All results are expressed as mean mg protein or DNA per whole brain $\pm$ S. E. (12).

Results :

As can be seen from Table 2, pups of the dams fed low protein diet during gestation (G^-) registered significant deficits in body weight and brain weight as compared to the controls (G^+) at birth, the deficits being 28 and 23 per cent respectively. The body weight deficits in G^+L^- group progressed from 41 per cent on day 7 to 59 per cent and day 14 and eventually to 70 per cent on day 21. Comparable deficits in G^-L^- group were 59, 70 and 73 per cent respectively. The brain weight deficits in G^+L^- group were 11, 26 and 20 per cent in G^-L^- group were 30, 42 and 25 per cent on day 7, 14 and 21 respectively. Although the brain weight to body weight ratio remained elevated in both the experimental groups, the elevation was more in G^-L^- group as compared to G^+L^- group till

day 14. The ratio was the same in both the groups on day 21.

Brain protein and DNA values showed significant reductions in both the undernourished groups at all ages studied (Table 3). However the reductions were more in G^-L^- group as compared to G^+L^- group. The deficits in protein and DNA contents in G^- group at birth were 25 and 20 per cent respectively. The deficits in brain protein content of G^+L^- group were 12, 26 and 27 per cent and of G^-L^- were 30, 44 and 32 per cent on day 7, 14 and 21, respectively. On these days the deficits in the DNA content of G^+L^- group were 16, 30 and 25 per cent and of G^-L^- group were 35, 46 and 31 per cent respectively. However, there was no significant difference in the protein to DNA ratio in any of the groups.

DISCUSSION :

The significant deficits observed in body and brain weights of G^- pups at birth are in agreement with that of other reports (16, 17). The elevated ratio of brain weight to body weight observed here is also comparable with the other report (18) and confirms the sparing effects observed for brain when an animal is submitted to early undernutrition (1). Body weight deficits in G^-L^- group were more as compared to G^+L^- group on days 7 and 14. However, this difference in body weight was not evident at day 21. Moreover, the differences in brain weight and also in the ratios of brain weight to body weight showed similar trend on day 21 in the present studies. However, these deficits were pronounced in the present studies as compared to other published reports (19, 20).

The significant decrease observed in brain protein and DNA at birth in $G^- L^-$ group can be attributed to more severe degree of undernutrition achieved in the present studies (19).

It is evident from brain DNA values that the production of cells in the control and both the undernourished groups had a similar pattern of development. The peak increment in DNA content was observed upto 7 days in all the groups (Table 3). However, both the undernourished groups had lower increment from birth to 7 days and 7 to 14 days. However, during the period between 14 and 21 days $G^- L^-$ group accumulated higher percentage of DNA than $G^+ L^+$ and $G^+ L^-$ groups. Similar acceleration is also observed in body and brain weights. Deo *et al* (21) have reported prolongation of the cell generation cycle and its G_2 and S phases in neonatally undernourished animals. Balazs *et al* (22) have reported significant prolongation in total cell cycle in brain upto 13 days of age in neonatally undernourished rats. However, significant prolongation of the total cell cycle was not observed at 21 days of age (22). In view of these reports, the accelerated accumulation of brain DNA observed in $G^+ L^+$ group between 14 to 21 days attains a special significance and may, perhaps, be corre-

lated with the changes in the cell cycle. However, similar degree of acceleration was not observed in $G^+ L^-$ group. This differential effect may be explained on the basis of timing and duration of undernutrition imposed on the progeny.

The values for brain protein content also showed a pattern similar to DNA content. It was consistently lower in $G^- L^-$ group as compared to $G^+ L^-$ group at all ages studied. It is interesting to note that $G^- L^-$ group registered higher accumulation of brain protein between 14 and 21 days. However, protein to DNA ratio was not affected in both the undernourished groups suggesting thereby that the cell size was not adversely affected.

In conclusion, maternal protein deficiency during gestation and lactation produces, in progeny, more drastic decrease in brain cell number as compared to maternal protein deficiency during lactation alone. However, brain cell size remained unaltered in both the experimental groups.

ACKNOWLEDGEMENTS :

These studies were partly funded by Grant-in-Aid Award to Dr. A. B. Kulkarni from Nutrition Society of India Hyderabad (NSI-79/60). We wish to thank Dr. A. J. Baxi for his interest in these studies.

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Synthesis and Study of 2-Substituted Alkyl and Aryl Amino-6-chlorobenzothiazoles as antituberculous agents

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In the present communication the appropriate 2-substituted alkyl- and aryl-amino-6-chlorobenzothiazoles were obtained by condensing 2-amino-5-chlorothiophenol with aryl or alkyl isothiocyanates, in nonpolar solvent at higher temperatures. The benzothiazoles were tested for their antituberculous activity against H₃₇Rv strain of *Mycobacterium tuberculosis* in vitro. Some of the compounds showed activity upto 3.12 g/ml.

Among the benzothiazoles, 2-substituted anilinobenzothiazoles are reported to be useful as bacteriocides and fungicides (1). Panitz *et al* (2) have recently reported significant anthelmintic activity for tioxidazole, a benzothiazole derivative.

Also, 2-substituted-anilinobenzothiazole and alkylaminobenzothiazoles reported from our laboratory, showed promising activity against *Mycobacterium tuberculosis* at the concentration of 3.12 mcg/ml in *in vitro* (3). In continuation of this it was of interest to establish a structure activity relationship among this type of derivatives. In this paper efforts have been made to synthesize different 6-chloro-2-substituted-aryl or alkyl-amino-benzothiazoles, and study them for anti-tuberculosis activity.

Chemistry :

Synthetic route consists of (1) preparation of 5-chloro-2-aminothiophenol, (2) synthesis of different alkyl and aryl

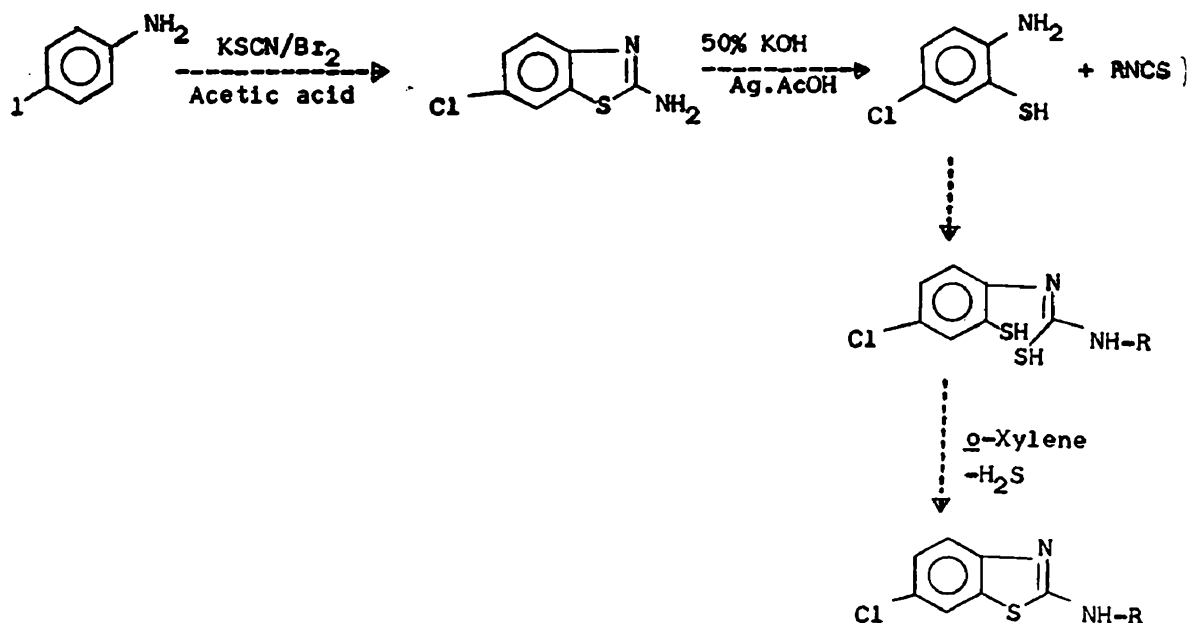
isothiocyanates, (3) condensation of 5-chloro-2-aminothiophenol with isothiocyanates (Scheme-I).

5-Chloro-2-aminothiophenol was obtained by alkaline hydrolysis of 2-amino-6-chlorobenzothiazole (4) and substituted-aryl and alkyl isothiocyanates were prepared by literature methods (5).

Biological Results and Discussion :

The *in vitro* antituberculosis activity was determined on *Mycobacterium tuberculosis* (H₃₇Rv) cultivated in Youmans' medium containing 10 per cent horse serum by a modified method of Doub and Youman (6).

Out of fifteen compounds, compounds Nos. 2, 4, 5, 7, 8 and 14 inhibited the growth of *M. tuberculosis* at 3.12 mcg/ml concentration ; while, Nos. 3, 7 and 16 were active at 6.25 mcg/ml concentration. Other compounds have the anti-TB activity at concentrations ranging from 12.5 mcg/ml to 100 mcg/ml.



R = Substituted-alkyl and aryl.

It has been observed that, all the 2-alkylamino compounds showed promising anti-TB activity. In the aryl-aminobenzothiazole series, the unsubstituted phenylamino derivative (No. 9) has activity upto 100 mcg/ml; however, when the substituent in the phenyl ring is electro-negative in nature like chloro or bromo, these compounds are inactive, while, when the substituents are like ethoxy or butoxy, the activity either goes on increasing with length of carbon chain or is retained.

Experimental Procedure :

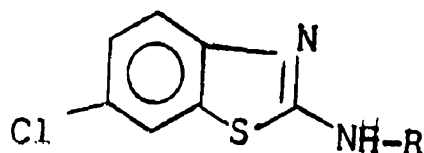
2-Amino-5-chlorothiophenol : A mixture of 2-amino-6-chlorobenzothiazole (9.23 g; 0.05 mole) and freshly prepared 50 per cent KOH solution (40ml) was taken in 100ml round bottomed flask equipped with water condenser. The flask was

heated in an oil-bath with occasional shaking for one hour or until evolution of ammonia gas ceased. The mixture was allowed to cool, diluted with water, and made slightly acidic at 0-5°C with 4N acetic acid. 2-Amino-5-chlorothiophenol was isolated by filtration and recrystallized from chloroform. Yield : 5.2 g (52.6%), m.p. 110°C (Lit. (4) m.p. 110°C).

2- (p-Chlorophenylamino) -6-chlorobenzothiazole : A mixture of 2-amino-5-chlorothiophenol (400 mg; 0.0025 mole) and p-chlorophenyl isothiocyanate (420 mg; 0.0025 mole) in o-xylene (25 ml) was refluxed for 4 hours in oil-bath. A solid began to separate during refluxing, the mixture was then cooled and filtered. The resultant anilino compound was recrystallized from ethanol. Yield : 400 mg (54.0%) ; m.p. 220°C.

TABLE — 1

2-Alkylamino and substituted-anilino-6-chlorobenzothiazoles.



where, R = Alkyl or substituted-aryl.

No.	R	Mol. formula	M. p.* °C	Yield %	Antitubercu- losis activity mcg/ml) **
1.	CH ₃	C ₈ H ₇ ClN ₂ S	195	45	12.5
2.	C ₂ H ₅	C ₉ H ₉ ClN ₂ S	125	53	3.12
3.	n-C ₃ H ₇	C ₁₀ H ₁₁ ClN ₂ S	75	58	6.25
4.	n-C ₄ H ₉	C ₁₁ H ₁₃ ClN ₂ S	100	62	3.12
5.	iso-C ₄ H ₉	C ₁₁ H ₁₃ ClN ₂ S	112	48	3.12
6.	Cyclo-C ₆ H ₁₁	C ₇ H ₁₅ ClN ₂ S	115	68	6.25
7.	CH ₂ CH=CH ₂	C ₁₀ H ₉ ClN ₂ S	105	43	3.12
8.	n-C ₆ H ₁₃	C ₁₃ H ₁₇ ClN ₂ S	102	62	3.12
9.	C ₆ H ₅	C ₁₃ H ₉ ClN ₂ S	190	52	100
10.	C ₆ H ₅ CH ₂	C ₁₄ H ₁₁ ClN ₂ S	203	68	Inactive
11.	4-BrC ₆ H ₄	C ₁₃ H ₈ BrClN ₂ S	221	53	"
12.	4-ClC ₆ H ₄	C ₁₃ H ₈ Cl ₂ N ₂ S	220	56	"
13.	4-CH ₃ OC ₆ H ₄	C ₁₄ H ₁₁ ClN ₂ OS	220	60	100
14.	4-C ₂ H ₅ OC ₆ H ₄	C ₁₅ H ₁₃ ClN ₂ OS	185	57	3.12
15.	4-C ₃ H ₇ OC ₆ H ₄	C ₁₆ H ₁₅ ClN ₂ OS	165	68	50
16.	4-C ₄ H ₉ OC ₆ H ₄	C ₁₇ H ₁₇ ClN ₂ OS	150	70	6.25
17.	H	C ₇ H ₅ ClN ₂ S	198†	70	6.25

* Melting points were taken in open capillary tubes and are uncorrected.

** Minimum inhibitory concentration *in vitro* anti-TB action for isoniazide = 0.04 mcg/ml ; and for p-Aminosalicylic acid = 0.4 mcg/ml.

† Reported m. p. (7).

Analysis for $C_{13}H_8Cl_2N_2S$:

Calcd. C, 52.86 ; H, 2.71 ; N, 9.48%

Found : C, 52.90 ; H, 2.42 ; N, 9.71%

All the anilino derivatives were prepared by a similar procedure and their relevant data reported in Table-1.

Acknowledgement :

The authors are thankful to Mr. M. T. Jaokar and his associates for micro-analyses. The authors are indebted to Dr. K. D. Sharma, Director, Haffkine Institute, Bombay 12, for providing excellent facilities to carry out the present work.

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Book-Review

Title : Antibiotic resistance: transposition and other mechanisms.

Eds : Mitsuhashi S., Rosival., Krcmery V., Cloth DM 98 Avicenum & Springer Verlag, ISBN 3-540-10322-8 Berlin Heidelberg New York.

The fourth international symposium on antibiotic resistance held at Smolenice, Czechoslovakia in June 1979 — the proceedings of which are reported in this volume — was mainly concerned with plasmid-mediated antibiotic resistance.

Though the introduction of potent antibiotics has revolutionised the chemotherapy of infectious diseases, it has brought fresh problems in its wake. The increasing prevalence of resistant bacteria has begun to cause problems not only in medicine, but also in fields like fish breeding and stock farming. As Mitsuhashi has pointed out in his introductory lecture, in large survey of resistant bacterial strains, R-plasmids are isolated from 60-80 per cent of the strains, explaining the preoccupation of this conference with plasmid-mediated resistance.

The first part of the volume contains papers dealing with theoretical aspects of plasmids and transposons, — the transferable genetic determinants for drug resistance carried by plasmids. Inoue *et al* have studied the genetic properties of nonconjugative plasmids which, so far, have not received as much atten-

tion as conjugative plasmids.

In the session on the plasmids of *Pseudomonas aeruginosa*, Holloway *et al* have discussed R-plasmids and bacterial chromosome transfer, while Riess *et al* and Michel-Briand *et al* have described plasmid variants. Work on *P-aeruginosa* R-plasmid gene mapping and translocation has been reported by Simon & Puhler and Lyobe *et al*).

In the session on transposons and replicons, analysis of plasmid genes responsible for resistance and for replication have been reported, with particular reference to gene amplification (by Schoffl) or recombination with bacteriophage P7 (by Mise) or to promoters of replication (by Ely *et al*). Mutants have been studied by Priefer *et al* and Watanbe *et al*.

The session on genetics and molecular biology once again has papers dealing mainly with genetic analysis of plasmids (Danbara *et al*, Vakulenko *et al*, Sova *et al*, and Van Treeck *et al*) or plasmid mutants (Ike *et al*, Nesvera *et al*, Schmidt *et al*). Interactions between plasmids and the host bacteria have been reported by Brana *et al*, Lebek *et al*, Roth *et al* and Hoffmeier *et al*.

In the session of plasmids in nature, Goebel *et al* and Yokota *et al* have reported studies with plasmids responsible for synthesis of χ -hemolysin and enterotoxin by *E. coli*. The paper by Lerin discusses the parameters that determine the fate of particular plasmid in bacterial

populations, while Cooke has reported studies on factor transfer under various environmental conditions such as shifts of temperature.

The second half of the proceedings deals mainly with medical implications. The first session of this part is devoted to discussion of new antibiotics like -1551 a semisynthetic Cephalosporin, (Matsubara *et al*), YM-09330 a semisynthetic cephamycin (Saito *et al*), Cefotaxime (Masuyoshi *et al*) and AM-715 a nalidixic acid analog (Ito *et al*). Ploczekova *et al* have reported results of preliminary immunochemical investigations of cephaloridine sensitive and resistant strains of *Salmonella* and Perea *et al* have studied the interactions of Azlocillin and Mezlocillin with aminoglycosides against *P. aeruginosa*.

In the second session, Delappe has outlined the scope of programmes of the National Institute of Health, USA for studying the phenomena in antibiotic resistance. The importance of transposons responsible for resistance to trimethoprim is underlined in the papers by Richards and Saroglou *et al*). The paper by Huges & Dutta described the distribution of gentamicin resistance plasmids in hospital infections. Resistance in *Bordetella bronchiseptica* has been studied by Terakado *et al*, and in *Serratia marcescens* by Eda *et al*.

The third session has papers concerned with structure and function of enzy-

mes that inactivate antibiotics (Gomez-Lus *et al*, Courvalin *et al*, Le Goffic *et al*, Kettner *et al* and Yokota *et al*). Biological properties of inactivation products of aminoglycoside antibiotics have been studied by Navashin *et al*.

The fourth session has discussed the epidemiology of resistant strains. The stability of such strains and spontaneous plasmid loss (Zueva *et al*) and the incidence of plasmids in pathogenic bacteria (Milch & Czironk, Hajinal *et al*, Palomares & Perea, Federic *et al* and Koltsida *et al*) were the subjects of discussion.

The last session has dealt with problems concerning surveillance of resistance. Suggestions regarding computerisation of clinical microbiology laboratory (Kayser *et al*) schemata for identifying resistance mechanisms from routine sensitivity screening results (O'Brien *et al*) and the problems of large-scale surveys (Grunt *et al* and Widemann) were among the topics discussed.

The first part of the proceedings that deals with the genetics and molecular biology of plasmids and their genes will be of interest, both for specific subjects and for an indication of the current state of the field, to bacterial geneticists working with R plasmids. The second part dealing as it does with matters of applied interest, will not doubt find a wide appreciative audience of medical microbiologists.

— R. B. Ghooi
Scientific Secretary

Bulletin of Haffkine Institute Vol. 9, 1981

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Annual Subscription : **Rs. 20 (Inland)** **Rs. 25 (Foreign)**

Edited and published by Dr. K. D. Sharma, Director, Haffkine Institute for Training Research and Testing, Bombay 400 012 and printed at Vijay Mudran, Bombay 400 031.

