

BULLETIN

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Natural Immunity Against Tetanus

Part I : A Study in Adivasi Population in Virar

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This problem was studied in an isolated and non-immunised Adivasi population living in a small town of Virar. Sera of thirty-three individuals were collected for antitoxin titration by passive mouse protection test and sixteen of them showed presence of antitoxin in the sera. Stool samples of the latter yielded growth of *Cl. tetani* in 13 individuals and 4 of the isolates were toxic and rest nontoxic. Isolation rate of the organism from faeces of horses and cows and from soil samples of that area varied from 72-83 per cent. These findings suggested that the population was living in a highly tetanus polluted area and some of them developed natural immunity against *Cl. tetani*. Probably, spores of the latter organism was ingested from the environment and the bacilli might have provided the necessary stimuli from the gut of the individuals to produce antibody or antitoxin in the sera.

Introduction

There is no doubt that there exists some correlation between the presence of tetanus bacilli in the digestive tract and tetanus antitoxin in blood (1,2). The latter probably results due to ingestion of tetanus spores from the environment and a long-delayed (3), continuous or intermittent liberation of sub-lethal doses of toxin in the intestinal tract which prove immunogenic enough to produce the antibody in the serum. This phenomenon might be producing natural immunity in 30-80% of the population living in contact with highly tetanus polluted areas and that may explain why

they give variable response to primary or booster dose of tetanus toxoid (4).

In India, tetanus antitoxin has been detected in non-immunized population (5,6) but there is hardly any information regarding the presence of *Cl. tetani* in environment or in human gut and its role in causing natural immunity. A preliminary work in this direction is presented in this article.

Materials and Methods

Reagents — Thioglycollate medium and Bacto-agar were of Difco, tetanus antitoxin (10 I.U./ml) and tetanus toxin were obtained from Haffkine Bio-Pharmaceutical Corp. Limt., Bombay.

Study area — This was located in a small town of Virar situated about 100 km north of Bombay and included 150-200 Adivasi population who were non-immunized and living in segregation at that place.

Samples — From thirty-three adults about 1-2 ml of serum was collected and stocked at -20°C till they were required for antitoxin titration. Stool of the individuals, cow and horses and soil samples of the area were collected separately in sterile plastic containers for anaerobic culture. No transport medium was used because the samples were processed within 2-3 hours after collection.

Culture procedures — A loopful of each stool sample was inoculated into Robertson cooked medium (RCM) containing 100 mcg/ml of neomycin. The medium prior to inoculation was heated for 10-15 minutes to drive out air, cooled rapidly, inoculated and sealed with a sterile layer of liquid paraffin to ensure perfect anaerobiasis. The tubes were incubated at 37°C for 7-10 days then subcultured in fresh medium and the process repeated till pure and adequate growth of anaerobes occurred in the RCM. One gram of soil sample was suspended in distilled water, heated at 80°C for 30 minutes and processed as stool samples.

Each sample was subcultured on two sets of thioglycollate agar plates, one was kept aerobically and the other anaerobically for 48 hours. Subsequently, isolation and identification of anaerobes

were done as procedures described elsewhere (7,8).

Toxicity of *Cl. tetani* isolates — A 7 days growth in RCM of the isolates was tested for toxicity in mice (9).

Titration of antitoxin in sera samples— This was determined by passive mouse protection test by technique employed by Massachusetts Public Health Laboratories, U.S.A. Initially Limus Tod (L+)/1000 level of toxin was determined against various dilutions of standard antitoxin. As antitoxin level in the population was expected to be less than 0.1 I.U./ml, so the standard antitoxin was diluted in peptone water to contain 0.005 I.U./ml. This was mixed with equal amount of various dilutions of toxin (1/1000-1/8000), mixture incubated at room temperature for 1 hour and 0.4 ml of each injected in a mouse (Table II). Average weight of mouse was 18-20 g, for each dose 3 animals were used and 2 kept as control. The latter were injected with equal volume of toxin and peptone water and the animals observed upto 4 days. The dilution that killed the animals between 72-96 hours was accepted as L+/1000 level of the toxin.

After this, antitoxin level in sera was determined by mixing L+/1000 dose of the toxin, serum and peptone water to get 0.0025, 0.005 and 0.01 antitoxin level/ml of each serum sample. Each of mixture was injected in 2 mice. Two animals served as a control and they were injected with a mixture of toxin and peptone water.

Table I
Isolation of *Cl. tetani*

Samples	Nos	Isolated		Nos	Animal toxicity
		Nos	%		%
Faeces-human	59	43	73	17	39.7
Faeces-Cow & horses	36	30	83	21	56.6
Soil	54	39	72	26	69

Table II
L+/1000 dose of toxin

Toxin dilution	Vol of toxin 1 ml (a)	Vol of antitoxin 1 ml (b)	Vol of (a) & (b) injected in mice 0.4ml	Death of mice in days			
				1	2	3	4
1/1000	"	"	"	DDD			
1/2000	"	"	"	DDD			
1/3000	"	"	"	DDD			
1/4000	"	"	"	DDD			
1/5000	"	"	"	D DD			
1/6000	"	"	"	DDD			
1/7000	"	"	"	D			
1/8000	"	"	"	000	000	000	000

D-indicates death of one or more animals, 0-no death.

Table III
Antitoxin titre in sera and isolation of *Cl. tetani*
in stool

Anaerobes isolated from stool	Antitoxin titre range I. U./ml	Numbers
Toxic <i>Cl. tetani</i>	—	0
	0.0025-0.01	0
	0.01 and greater	4
Non-toxic <i>Cl. tetani</i>	—	3
	0.0025-0.01	3
	0.01 and greater	6
Other anaerobes not identifiable	—	10
	0.0025-0.01	3
	0.01 and greater	0
samples not available	—	4
Total		33

Results

Isolation rate and toxicity of *Cl. tetani* isolates — The isolation rate varied from 72-83% (Table I) and they were more frequently isolated from animal faecal matter than human beings and soil samples. The number of isolates from cow and horses did not vary to a significant number, so they have been kept together. The same table shows that isolates from soil proved toxic in greater number than others.

Level of antitoxin in sera samples — L+/ 1000 dose of the toxin was represented by 1/4000 dilution which killed mice in 72-96 hours. In all thirty-three sera were tested and sixteen (48%) showed presence of antitoxin. From twenty-nine stool samples, sixteen

(55%) were positive for *Cl. tetani* and thirteen such individuals showed presence of antitoxin in their sera (Table III). As regards toxicity, more isolates were non-toxic but persons passing toxic strains in stool showed a higher titre (0.01 I. U./ml) of antitoxin than the former. This suggested that toxigenic strains possessed greater immunogenic property than the non-toxic ones.

Discussion

The concept of natural immunity against tetanus originated from the work of Tenbroeck and Baur (1) who detected presence of tetanus antitoxin in 37.5% of non-immunized population who excreted *Cl. tetani* in stool. Later, other workers, (2, 10) showed that the natural immunity depended upon the pollution rate

of the environment by *Cl. tetani* being higher in population living in contact with a highly tetanus — polluted area. In this study, Adivasi population lived in such an area and it was not surprising that out of 33 individuals, 48% of them showed presence of antitoxin in sera and most of them excreted *Cl. tetani* in faeces. Among the isolates most were non-toxigenic for mice. This suggested that both toxigenic and non-toxigenic

strains of *Cl. tetani* could provide the necessary stimuli from the intestine of the individuals to produce antibody or antitoxin in sera of the individuals. This requires further study for confirmation.

Acknowledgements

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Serum Antibody Response in Albino Rats after the Transfer of Adult *Litomosoides carinii* Worms

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Adult *Litomosoides carinii* worms, 6 to 8 per rat, were transplanted intraperitoneally. The worms were transplanted sex wise in four groups: (A) Female worms; (B) Male worms; (C) Worms of both sexes and (D) Control. Haemagglutinin and precipitin types of antibodies were examined in all the groups. The haemagglutinin antibody titre was found to increase in rats which received adult female worms or both male and female worms as compared to that in rats having male worms only. Precipitin line due to counter immunoelectrophoresis test is predominant in rats belonging to groups A and C. This investigation suggests that there is an extra elicitation of antigenic source present in female worms due to microfilariae in the circulating blood.

Introduction

In cotton rat filariasis infection produced by the filariid worm *Litomosoides carinii*, the elevation of serum antibody level depends on factors like the number of worms parasitising the host, antigenic potency of various developing stages and microfilarial density in the host blood (1,2,3). It has been demonstrated that the serum antibodies rise much earlier in cotton rats transplanted with *L. carinii* worms in comparison to rats exposed to infective larvae (4, 5). However, these observations refer mainly to cotton rat — *L. carinii* system. There is dearth of information on pattern of antibody production when albino rat — *L. carinii* system is considered. Many investigators favour the use of albino rat — *L. carinii* model for immunological investigations. Therefore, it was considered worthwhile to

take up a study to investigate antibody pattern in albino rats transplanted with filariid worms. The following account describes our findings.

Materials and Methods

Eight *L. carinii* infected rats, three month old, showing microfilaremia of approximately 2000/10 cmm of blood were selected as donor rats. These animals were killed by ether anaesthesia and adult *L. carinii* worms were gently removed with the help of forceps from the thoracic cavity. These worms were washed thrice in sterile saline and were transplanted into the abdominal cavity of the rat (6, 7). Twenty-four male rats were grouped into four groups of 6 each. Each group received worms as per description given below :

Antibody Response to *L. carinii*

Group	No. of male rats (Body wt. 100-120 gm)	Male/female worms transplanted into peritonium of rat
A	6	6-8 female worms
B	6	6-8 male worms
C	6	4 male + 4 female worms
D	6	Uninfected (Control)

In the recipient rats, the incision was sutured with silk thread and later the site was dusted with nebasulph powder. Recipient animals, at least two or three rats, were bled every week either by cardiac puncture or by snipping the tail for obtaining the serum preparation and preparing blood films.

Antigen was prepared by using fresh, active adult worms after washing them several times with merthiolated saline and phosphate buffer saline (pH 7.2). These worms were sonicated at 4°C. The suspension was centrifuged at 5000 rpm for 30 minutes at 4°C and protein value of the supernatant was determined (6.5 mg/ml) by Lowry's method (8). The antigen was stored at -20°C for use.

Indirect haemagglutination test was performed on microtitre plates by using 2% sheep RBC which were already preserved in Alsever's solution and washed with normal saline and PBS. The erythrocytes were tanned with Tanic acid (1:20000) in PBS (pH 7.2). Diluted antigen (5 times) was selected for serology after titrating the antigen against positive serum. Both positive and negative controls were kept along with test sera.

Counterimmunoelectrophoresis was performed in two agar plates (7.5 x 2 cms.), each was prepared with 0.75% Nobel agar in veronal buffer (pH 8.4) to which Merthiolate (1:10000) was added. The wells were spaced 5 mm apart and 6 samples were run on each plate (9). Electrophoresis was carried out at 200 volts for 1 hour at 10°C. Slides were washed with normal saline and the dried slides were stained with Amido black (1% Amido Schwartz in solution containing methanol, acetic acid and distilled water in ratio 40:10:50), de-colourised in 2% acetic acid and finally dried at room temperature.

Results**Recovery of the transplanted worms:**

Worms recovered from animals at autopsy from day 0 to 16 post transplantation were found alive while dead and encapsulated worms were recovered thereafter.

Microfilaria counts: The microfilariae were observed in the blood of recipient rats (groups A and C) during 15 days post transplantation period. No microfilariae were observed in group B and group D (control) animals.

Haemagglutination test: The titre of haemagglutinating antibody in group A rats

Table 1

Development of serum antibodies in rat sera after transfer of adult *L. carinii* worms to their peritoneal cavities.

Group	Sex of worms transferred	Serological Test	Post transfer period in weeks							
			1	2	4	6	8	10	12	14
A	Female worms	IHA Titre	1:256	1:512	1:512	1:512	1:16	1:16	1:16	1:16
		CIEP value	—	+	+	+	+	—	—	—
B	Male worms	IHA Titre	—	—	1:256	1:8	1:8	1:8	1:4	1:16
		CIEP value	—	—	—	+	—	—	—	—
C	Female and male worms	IHA Titre	1:256	1:512	1:512	1:16	1:16	1:4	—	—
		CIEP value	—	+	+	+	—	—	—	—
D	Control Uninfected	IHA Titre	—	—	—	—	—	—	—	—
		CIEP value	—	—	—	—	—	—	—	—

- initially showed 1 : 256 in the 1st week which increased to 1 : 512 and remained constant until the 6th week. This titre dropped to 1 : 16 from the 8th week onwards. Similar pattern was also observed in group C till the 4th week post transplantation. In group B rats such serum antibodies were not detected in the first two weeks. The antibody appearance was prolonged up to the 4th week, titre showing 1 : 256, followed by a significant drop in the later period of infection (Table 1).

Counterimmunoelectrophoresis : Female worm-recipient rat sera in group A showed precipitin line against antigen from the 2nd week to 8th week infection period. Similar observation was also noted in the rats of group C which were the recipients of male and female worms. Group B consisting of male worms recipient rats were negative throughout the experimental period except in the 8th week (Table 1).

Discussion

The implanted *L. carinii* worms in the abdominal cavity of normal albino rats are found to survive for 15 days and microfilariae are seen in the blood. Implantation of worms brings about the pro-

duction of high antibody level ; both haemagglutinin and precipitin types are observed in the sera of recipient rats. This indicates an extra elicitation of antigenic material by female worms and so also by circulating microfilariae. Our study is in agreement with the findings of other workers who suggested that the stimuli for production of antibodies were generated principally by adult worms (1, 2, 4, 5). In the female worm recipient rats the haemagglutinin or precipitin antibodies remained to persist as long as 6 to 8 weeks after transplantation of the worms. However, these antibodies were not observed significantly in male worm recipient albino rats. Such antibodies after transplantation of worms began to appear much earlier than the normal course of *L. carinii* infection in cotton rats (10, 11). The antibodies, however, showed till 11.5 weeks and disappeared after 13 weeks (5).

It is suggested that the female worm antigenic material is highly potent and plays an important role in acquired resistance to reinfection (10, 11). Our study also supports the observations that female adult worms stimulate humoral immunity in *L. carinii* infection.

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Synthesis and Study of 5(6)-Substituted-2-(Substituted Benzyl)Thio Sulfonyl Benzimidazoles as possible Antituberculosis Agents

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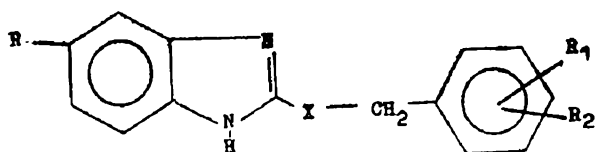
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A series of 5(6)-substituted-2-(substituted benzyl)-thio benzimidazoles have been synthesised and some of them oxidised to the corresponding sulfones with potassium permanganate. The compounds were screened for *in vitro* anti T.B. activity against *M. tuberculosis* (H₃₇Rv strain).

Introduction

A number of benzimidazole derivatives (1) possess a variety of biological actions, such as anthelmintic, antitubercular and antimalarial.

Mayer's hypothesis (2) postulates that the mycobacteria and fungi being phylogenitically related, substances acting as antifungal agents are likely to show antituberculous activity also. The present work was prompted due to the reported (3) antifungal activity of 5(6)-chloro-2-benzyl thiobenzimidazole. Also, in view of the well known properties of sulfones as antileprotic and tuberculostatic compounds the synthesis of the title compounds shown below was undertaken with a view of studying their anti-tuberculous activity.



where,

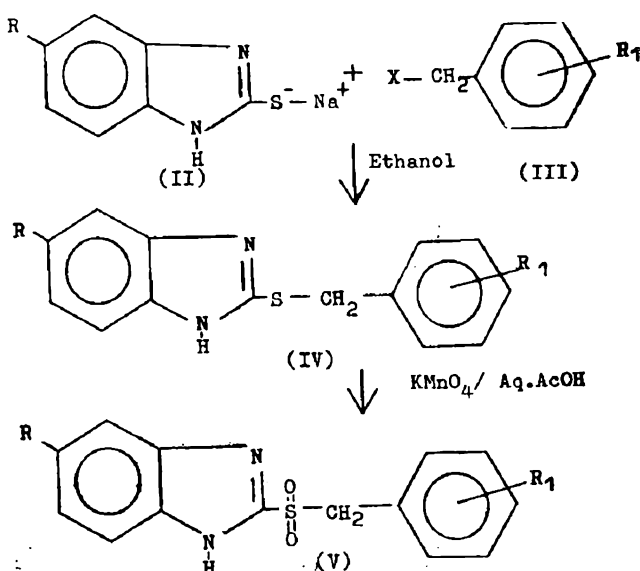
X = S, SO₂; R = H, Cl, CH₃, NO₂;

R₁ = H, 2-Cl, 3-Cl, 2-OH, and

R₂ = 4-Br, 4-Cl, 5-NO₂.

Chemistry

The proposed compounds (IV) were prepared by reacting sodium salt of 5(6)-substituted - 2 - mercapto - benzimidazo-



les (II) with different substituted benzyl halides (III) in boiling ethanol and some of them were further oxidised to sulfones (V) by potassium permanganate in aqueous acetic acid.

The 5(6)-substituted-2-mercapto-benzimidazoles were prepared by general method (5). Substituted benzyl halides were obtained by the side-chain bromina-

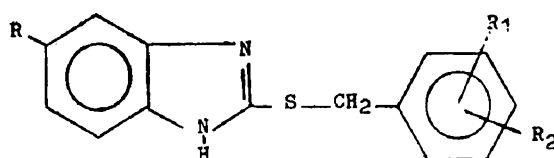
tion of the corresponding toluenes at higher temperature and in the presence of light as described in literature (6).

Experimental :

The general method for the preparation of 5(6)-substituted-2-(substituted benzyl)thio sulfonyl benzimidazoles is illustrated by the following typical experiments.

Table — I

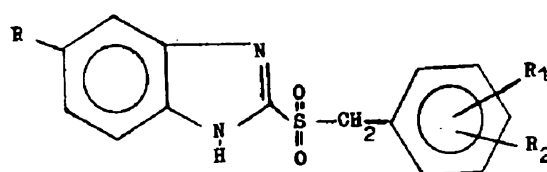
5(6)-Substituted-2-(substituted-benzyl)thio, benzimidazoles.



No.	R	R ₁	R ₂	M.P. °C ^a (uncorr.)	Mol. formula ^b	M. I. C. ^c (mcg/ml)
1.	Cl	2-Cl	4-Cl	177	C ₁₄ H ₉ Cl ₃ N ₂ S	Inactive
2.	Cl	3-Cl	4-Cl	185	C ₁₄ H ₉ Cl ₃ N ₂ S	100
3.	Cl	2-OH	5-NO ₂	240	C ₁₄ H ₁₁ ClN ₃ O ₃ S	10
4.	Cl	H	4-Br	198	C ₁₄ H ₁₁ BrClN ₂ S	200
5.	Cl	2-Cl	4-NO ₂	223	C ₁₄ H ₉ Cl ₂ N ₃ OS.HCl	1.56
6.	Cl	2-Br	4-NO ₂	230	C ₁₄ H ₉ BrClN ₃ OS	25
7.	CH ₃	3-Cl	4-Cl	197	C ₁₅ H ₁₂ Cl ₂ N ₂ S	2
8.	CH ₃	2-Cl	4-Cl	160	C ₁₅ H ₁₂ Cl ₂ N ₂ S	50
9.	CH ₃	2-OH	5-NO ₂	292	C ₁₅ H ₁₃ N ₃ O ₂ S	25
10.	CH ₃	H	4-Br	189	C ₁₅ H ₁₃ BrN ₂ S	Inactive
11.	CH ₃	2-Cl	4-NO ₂	134	C ₁₅ H ₁₂ ClN ₃ O ₂ S	200
12.	CH ₃	2-Br	4-NO ₂	152	C ₁₅ H ₁₂ BrN ₃ O ₂ S	100
13.	NO ₂	3-Cl	4-Cl	140	C ₁₄ H ₉ Cl ₂ N ₃ O ₂ S	50
14.	NO ₂	2-Cl	4-Cl	137	C ₁₄ H ₉ Cl ₂ N ₃ O ₂ S	3.12
15.	NO ₂	2-OH	5-NO ₂	227	C ₁₄ H ₁₀ N ₄ O ₅ S	Inactive
16.	NO ₂	H	4-Br	147	C ₁₄ H ₁₀ BrN ₃ O ₂ S	25
17.	NO ₂	2-Cl	4-NO ₂	162	C ₁₄ H ₉ ClN ₄ O ₄ S	25
18.	NO ₂	2-Br	4-NO ₂	210	C ₁₄ H ₉ BrN ₄ O ₄ S.HCl	12.5

Table — II

5(6) -Substituted-2- (substituted-benzyl) sulfonyl, benzimidazole.



No.	R	R ₁	R ₂	M.P.°C ^a (uncorr.)	Mol. formula ^b	M.I.C. ^c (mcg/ml)
1.	Cl	2-Cl	4-Cl	210	C ₁₄ H ₉ Cl ₃ N ₂ O ₂ S	2
2.	Cl	H	4-Br	200	C ₁₄ H ₁₁ BrClN ₂ O ₂ S	5
3.	Cl	2-OH	5-NO ₂	210	C ₁₄ H ₁₁ ClN ₃ O ₅ S	100
4.	Cl	2-Cl	4-NO ₂	212	C ₁₄ H ₉ Cl ₂ N ₃ O ₄ S	1.56
5.	CH ₃	H	4-Br	237	C ₁₅ H ₁₃ BrN ₂ O ₂ S	100
6.	CH ₃	3-Cl	4-Cl	186	C ₁₅ H ₉ Cl ₂ N ₂ O ₂ S	5
7.	CH ₃	2-Cl	4-Cl	205	C ₁₅ H ₉ Cl ₂ N ₂ O ₂ S	2
8.	NO ₂	2-Cl	4-Cl	195	C ₁₄ H ₉ Cl ₂ N ₃ O ₄ S	50
9.	NO ₂	3-Cl	4-Cl	185	C ₁₄ H ₉ Cl ₂ N ₃ O ₄ S	12.5
10.	NO ₂	2-Cl	4-NO ₂	167	C ₁₄ H ₉ ClN ₄ O ₆ S	25
11.	NO ₂	2-Br	4-NO ₂	77	C ₁₄ H ₉ BrN ₄ O ₆ S	25

(a) Melting points taken in open capillary tubes and are uncorrected.

(b) All compounds were analysed for C, H and N and the values were within $\pm 0.4\%$.(c) Minimum inhibitory concentration for *in vitro* antituberculosis activity (for INH = 0.04 mcg/ml and for PAS = 0.4 mcg/ml).

5 (6)-Nitro-2-(2,4-dichlorobenzyl) thio benzimidazole :

A mixture of 2.17 g of sodium salt of 5(6)-nitro-2-mercapto benzimidazole (0.01 mole) and 2.40 g of 2,4-dichlorobenzyl bromide (0.01 mole) taken in 30 ml of ethanol and refluxed on steam-bath for 3 hrs. The solvent was completely removed under reduced pressure. Some water was added, and the precipitate filtered and crystallised from aqueous ethanol. Yield 92.0%, mp. 137°C.

5(6)-Nitro-2-(2,4-dichlorobenzyl) sulfonyl benzimidazole :

About 1.15 g of 5(6)-nitro-2-(2,4-dichlorobenzyl)-thio benzimidazole (0.005 mole) was dissolved in a minimum amount of glacial acetic acid (about 20 ml) by warming on water-bath. Then, a 7% aqueous solution of KMnO_4 was added gradually at 30-40°C. As the oxidation proceeded and the pink colour of KMnO_4 solution was discharged and further KMnO_4 solution was added. This was continued till the KMnO_4 colour persisted. After allowing the reaction mixture to cool overnight, NaHSO_3 was added to decompose the excess of

KMnO_4 used. The mixture was poured into ice-water, when the sulfonyl derivative precipitated. It was filtered, washed with water and crystallised from ethanol. Yield : 48.0%; m.p. 195°C.

Biological Results :

All the compounds synthesised were tested for their *in vitro* antituberculosis activity against human virulent strain of *M. tuberculosis* (H₃₇Rv strain) by a serial dilution method of Doub and Youmans (7).

Out of eighteen sulfide derivatives tested, nine compounds showed activity upto 40 mcg/ml at minimum inhibitory concentration, whereas the highest activity exhibited at 1.56 mcg/ml conc. by compound No. 5 (Table I).

Among the eleven sulfonyl derivatives tested, six compounds showed activity at 40 mcg/ml. conc. Compound No. 4 (Table II) inhibited the growth of *M. tuberculosis* at 1.56 mcg/ml conc. Some of the sulfides showed better activity than their corresponding sulfones and vice versa.

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Synthesis and Study of Schiff Bases Derived from 2-(4-Aminophenyl) Thiazoles for In Vitro Antituberculosis Activity - 2

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Schiff bases from physiologically important aromatic aldehydes and a few -(4-aminophenyl) thiazoles, which showed *in vitro* antituberculosis activity, have been prepared and tested for *in vitro* antituberculosis activity. A number of these compounds displayed fairly significant *in vitro* activity and three Schiff bases were found to be active as thioacetazone under identical condition of *in vitro* testing.

Introduction

Schiff bases, as has been proved, form a class of important compounds in medicinal and pharmaceutical fields. Three drugs used for the treatment of tuberculosis belong to this class of compounds, viz., thioacetazone, rifampicin and phthivazid (1). In addition, compounds belonging to this class have also shown antibacterial (nitrofurazone, furazolidone and nitrofurantoin), antiviral (N-substituted-isatin- β -thiosemicarbazones) (2), antimalarial and antileprotic properties (3).

Schiff base of 4-acetamidobenzaldehyde, thioacetazone, is an important drug in the treatment of tuberculosis. Schiff bases from 5-chlorosalicylaldehyde, 5-bromosalicylaldehyde, 4-N, N-dimethylaminobenzaldehyde have been reported to show encouraging tuberculostatic activity (4). The thiosemicarbazone, a

Schiff base, of indole-3-aldehyde has shown good activity against *Mycobacterium tuberculosis* and has also been found to be active subcutaneously in mice (5).

We have already reported the *in vitro* antituberculosis activity of 2-(4'-aminophenyl)thiazoles (6, 7).

Therefore, it was felt that if we could combine the potentially active 2-(4'-aminophenyl)thiazoles (I) and other active aldehydes (II, III and IV) in the form of Schiff bases, the resultant compounds may show better activity. With this view, we have prepared Schiff bases (V, VI, and VII) by condensing thiazolyl amines (I) physiologically important aldehydes (II, III and IV).

Chemistry

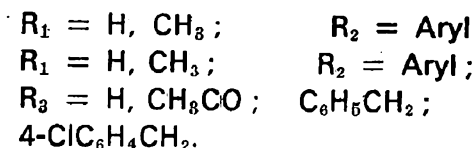
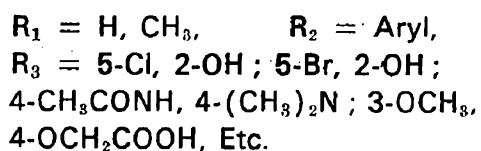
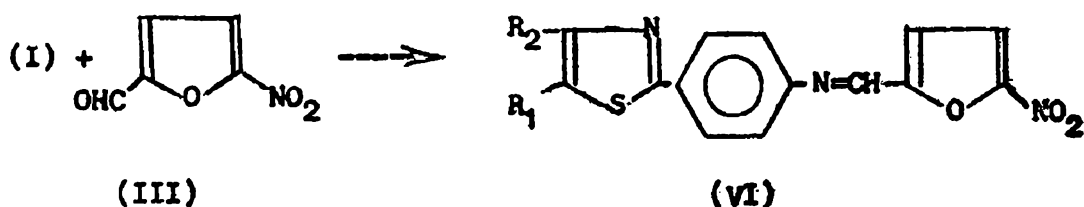
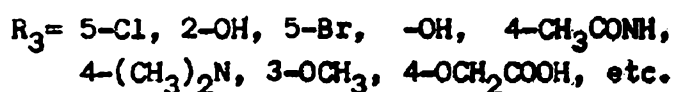
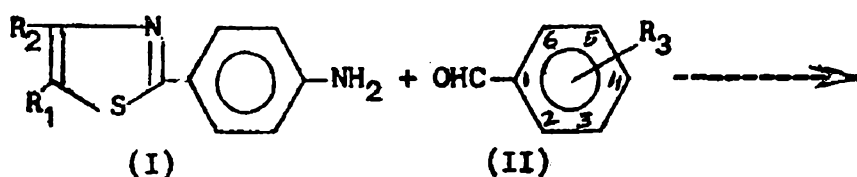
The synthesis of Schiff bases (V, VI, VII; Tables I to III) was brought about

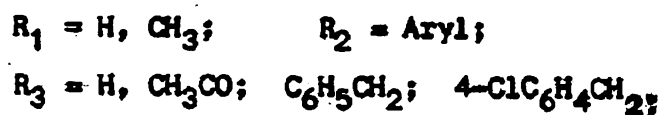
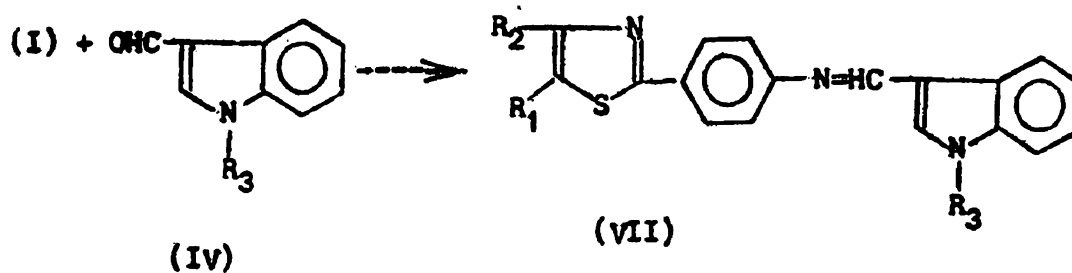
by refluxing the required 2-(4-aminophenyl)thiazole (I) or its hydrochloride and the requisite aldehyde (II) in alcohol. The required benzaldehydes (II) were prepared according to methods cited (8-13).

Indole, on treatment with dimethylformamide (DMF) and phosphorus oxychloride under the reaction conditions of Villsmeyer and Haack, gave indole-3-al-

dehyde (14) (IV) and further reaction with acetic anhydride, benzyl chloride and 4-chlorobenzyl chloride gave N-acetyl (15), N-benzyl (16) and N-(4'-chlorobenzyl) derivatives thereof.

The requisite 2-(4'-aminophenyl)thiazoles were prepared by the condensation of different haloketones with 4-aminothiobenzamide (6, 7).





Experimental

N-(4-Chlorobenzyl)indole-3-aldehyde :

Indole, 3-aldehyde (14.5 g ; 0.1 mole), 4-chlorobenzyl chloride (17.7 g ; 0.11 mole), anhydrous potassium carbonate (13.8 g ; 0.1 mole) and DMF (29.2 g ; 0.4 mole) were heated on a steam-bath with vigorous stirring for two hours. After cooling to room temperature, the solution was poured into water (120 ml) and the precipitated solid was filtered, dried and recrystallized from ethanol to give crystals of N-(4-chlorobenzyl)-indole, 3-aldehyde.

Yield : 24.0 g (89.0%) ; m.p. 118-119°C.

Anal. calcd. for $\text{C}_{16}\text{H}_{12}\text{ClNO}$:

Found :
 C, 44.94 ; H, 4.45 ; N, 5.19%
 C, 44.70 ; H, 4.61 ; N, 5.01%.

2-(4-(4-(Acetamidobenzylidene)amino)phenyl)-4-(4'-chlorophenyl)thiazole hydrochloride :

To a solution of 2-(4'-aminophenyl)-4-(4'-chlorophenyl)-thiazole hydrochloride (0.64 g ; 0.002 mole) in ethanol was added a hot solution of 4-acetamidoben-

zaldehyde (0.32 g ; 0.002 mole) in ethanol. The resulting yellow solution was refluxed for 15 minutes. On cooling to room temperature, it deposited a yellow crystalline solid. This was collected by filtration, washed with boiling ethanol (20 ml) and then with a little diethyl ether to obtain the desired Schiff base, as its monohydrochloride salt.

Yield : 0.8 g (85.0%) ; m.p. 253-255°C.

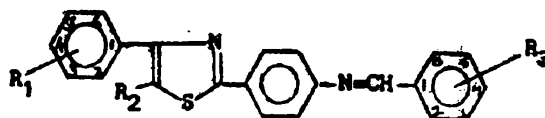
Anal. calcd. for $\text{C}_{24}\text{H}_{18}\text{ClN}_3\text{OS} \cdot \text{HCl}$:

Found :
 C, 61.55 ; H, 4.06 ; N, 8.97%
 C, 61.21 ; H, 3.96 ; N, 8.74%.

2-(4-(3-(N-Benzyl)indolidene)amino)phenyl)-4-(4'-fluorophenyl)thiazole hydrochloride :

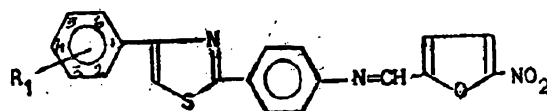
To a solution of 2-(4'-aminophenyl)-4-(4'-fluorophenyl) thiazole hydrochloride (0.31 g ; 0.001 mole) in ethanol was added a hot solution of N-benzylindole, 3-aldehyde, (0.24 g ; 0.001 mole) in ethanol. The resulting solution was refluxed for 20 minutes. On cooling to room temperature, it deposited a crystalline solid. This was collected by filtration, washed with ethanol (10 ml) and then with a little diethyl ether to give

Table-I
Schiff bases from 2-(4-aminophenyl) thiazoles



No.	R	R ₁	R ₂	M.P.°C (uncorr)	Mol. formula	Minimum inhibitory concentra- tion (mcg/ml)
1.	4-Cl	H	4-NHCOCH ₃	253-55	C ₂₄ H ₁₈ ClN ₃ OS.HCl	10
2.	4-Br	H	"	280-82	C ₂₄ H ₁₈ BrN ₃ OS.HCl	10
3.	4-F	H	"	259-61	C ₂₄ H ₁₈ FN ₃ OS.HCl	5
4.	4-CH ₃	H	"	268-70	C ₂₅ H ₂₁ N ₃ OS.	100
5.	—	CH ₃	"	255-57	C ₂₅ H ₂₁ N ₃ OS.HBr	10
6.	3-CH ₃ CO- NH-4-CH ₃ O	H	"	234-36	C ₂₇ H ₂₄ N ₄ O ₃ S.HCl	5
7.	4-Cl	H	4-N (CH ₃) ₂	235-36	C ₂₄ H ₂₀ ClN ₃ S	20
8.	4-Br	H	"	273-74	C ₂₄ H ₂₀ BrN ₃ S.HCl	5
9.	4-F	H	"	243-45	C ₂₄ H ₂₀ FN ₃ S.HCl	10
10.	4-CH ₃	H	"	209-10	C ₂₅ H ₂₃ N ₃ S	5
11.	—	CH ₃	"	182-84	C ₂₅ H ₂₃ N ₃ S	40
12.	3-CH ₃ CO- NH-4-CH ₃ O	H	"	204-06	C ₂₇ H ₂₆ N ₄ O ₂ S	5
13.	4-Cl	H	2-OH,5-Cl	253-54	C ₂₂ H ₁₄ Cl ₂ N ₂ OS	200
14.	4-Br	H	"	263-64	C ₂₂ H ₁₄ BrClN ₂ OS	Inactive
15.	4-F	H	"	229-30	C ₂₂ H ₁₄ ClFN ₂ OS	"
16.	4-CH ₃	H	"	265-66	C ₂₃ H ₁₇ ClN ₂ OS	"
17.	—	CH ₃	"	179-80	C ₂₃ H ₁₇ ClN ₂ OS	100
18.	3-CH ₃ CO- NH-4-CH ₃ O	H	"	229-31	C ₂₅ H ₂₀ ClN ₃ O ₃ S	100
19.	4-Cl	H	2-OH,5-Br	264-65	C ₂₂ H ₁₄ BrClN ₂ OS	Inactive
20.	4-Br	H	"	271-72	C ₂₂ H ₁₄ Br ₂ N ₂ OS	"
21.	4-F	H	2-OH,5-Br	237-39	C ₂₂ H ₁₄ BrFN ₂ OS	Inactive
22.	4-CH ₃	H	"	269-71	C ₂₃ H ₁₇ BrN ₂ OS	"
23.	—	CH ₃	"	189-90	C ₂₃ H ₁₇ BrN ₂ OS	"
24.	3-CH ₃ - CONH,4-CH ₃ O	H	"	235-36	C ₂₅ H ₂₀ BrN ₃ O ₃ S	100
25.	4-C ₂ H ₅ O	H	"	250-51	C ₂₄ H ₁₈ BrN ₂ O ₂ S.HCl	—
26.	4-Cl	H	3-OCH ₃ ,4- OCH ₂ COOH	238-40	C ₂₅ H ₁₈ ClN ₂ O ₄ S.HCl	2
27.	4-Br	H	"	244-46	C ₂₅ H ₁₉ BrN ₂ O ₄ S.HCl	2
28.	4-F	H	"	220-22	C ₂₅ H ₁₉ FN ₂ O ₄ S.HCl	5
29.	4-CH ₃	H	"	174-75	C ₂₆ H ₂₂ N ₂ O ₄ S	5
30.	3-CH ₃ CO- NH,4-CH ₃ O	H	"	125-26	C ₂₈ H ₂₅ N ₃ O ₃ S	2

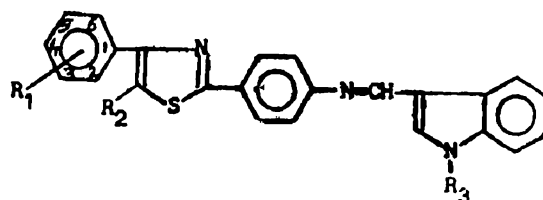
Table-II
Schiff bases from 2-(4-aminophenyl)thiazoles.



No.	R ₁	M. p. °C (uncorr)	Mol. formula	Minimum inhibitory concentration (mcq/ml)
31.	4-Cl	149-51	C ₂₀ H ₁₂ ClN ₃ O ₃ S	5
32.	4-Br	240-41	C ₂₀ H ₁₂ BrN ₃ O ₃ S	5
33.	4-F	225-27	C ₂₀ H ₁₂ FN ₃ O ₃ S	40
34.	4-CH ₃	229-30	C ₂₁ H ₁₅ N ₃ O ₃ S	Inactive
35.	3-CH ₃ CONH,4-CH ₃ O	264-66	C ₂₃ H ₁₆ N ₄ O ₅ S. HCl	—

Table-III

Schiff bases from 2-(4-aminophenyl)thiazoles.



No.	R ₁	R ₂	R ₃	M. p. °C ^a (uncorr)	Mol. formula ^b	Minimum inhibitory concentration (mcg/ml) ^c
36.	4-Cl	H	H	240-42	C ₂₄ H ₁₆ ClN ₃ S	10
37.	4-Br	H	H	244-45	C ₂₄ H ₁₆ BrN ₃ S	100
38.	4-F	H	H	277-78	C ₂₄ H ₁₆ FN ₃ S.HCl	5
39.	4-CH ₃	H	H	215-16	C ₂₅ H ₁₉ N ₃ S	10
40.	—	CH ₃	H	162-65	C ₂₅ H ₁₉ N ₃ S	10
41.	3-CH ₃ CO- NH,4-CH ₃ O	H	H	205-06	C ₂₇ H ₂₂ N ₄ O ₂ S	5
42.	4-Cl	H	COCH ₃	204-05	C ₂₆ H ₁₈ ClN ₃ OS	100
43.	4-Br.	H	COCH ₃	205-06	C ₂₆ H ₁₈ BrN ₃ OS	40
44.	4-F	H	COCH ₃	266-69	C ₂₆ H ₁₈ FN ₃ OS.HCl	5
45.	4-CH ₃	H	COCH ₃	120-22	C ₂₇ H ₂₁ N ₃ OS	40
46.	—	CH ₃	COCH ₃	161-63	C ₂₇ H ₂₁ N ₃ OS	40
47.	4-Cl	H	CH ₂ -Ph	195-96	C ₃₁ H ₂₂ ClN ₃ S	40
48.	4-Br	H	CH ₂ -ph.	201-02	C ₃₁ H ₂₂ BrN ₃ S	40
49.	4-F	H	CH ₂ Ph	259-61	C ₃₁ H ₂₂ FN ₃ S.HCl	5
50.	4-CH ₃	H	CH ₂ Ph	141-43	C ₃₂ H ₂₅ N ₃ S	5
51.	—	CH ₃	CH ₂ Ph	107-09	C ₃₂ H ₂₅ N ₃ S	10
52.	4-Cl	H	p-ClC ₆ H ₄ CH ₂	175-77	C ₃₁ H ₂₁ Cl ₂ N ₃ S	40
53.	4-Br	H	p-ClC ₆ H ₄ CH ₂	183-85	C ₃₁ H ₂₁ BrClN ₃ S	40
54.	4-F	H	p-ClC ₆ H ₄ CH ₂	274-77	C ₃₁ H ₂₁ ClFN ₃ HCl	40
55.	4-CH ₃	H	p-ClC ₆ H ₄ CH ₂	143-44	C ₃₂ H ₂₄ ClN ₃ S	40
56.	—	CH ₃	p-ClC ₆ H ₄ CH ₂	236-38	C ₃₂ H ₂₄ ClN ₃ S	40

(a) Melting points are capillary melting points and are uncorrected.

(b) Analysed correctly for C, H and N within $\pm 0.4\%$.(c) Minimum inhibitory concentration for *in vitro* antituberculosis action for isoniazid = 0.04 mcg/ml, for p-aminosalicylic acid = 0.4 mcg/ml and thioacetazone = 2.0 mcg/ml.

the desired Schiff base as its monohydrochloride salt.

Yield : 0.38 g (73.0%) ; m.p. 259-261°C.

Anal. calcd. for $C_{31}H_{22}FN_3S \cdot HCl$:

Found :
C, 71.06 ; H, 4.39 ; N, 8.02%
C, 70.75 ; H, 4.64 ; N, 7.78%.

Discussion

Out of 56 Schiff bases prepared during the course of this work, 54 have been tested in *vitro* system by using the serial dilution method of Doub and Youmans (17) against the human virulent strain (H_3R_v) of *Mycobacterium tuberculosis*. Twenty-one Schiff bases were active below 40 mcg/ml and seventeen were active below 5 mcg/ml.

Three Schiff bases (V, Compounds No. 26, 27, 30 and Table I) were active

at 2 mcg/ml, this activity being comparable with that of antituberculosis drug thioacetazone having azomethine linkage under identical conditions of in *vitro* testing.

Acknowledgement

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Synthesis and Screening for Antitubercular activity of Substituted-S-(Pyrimidyl or Quinolyl)-2-(Thio or Sulfonyl) Benzimidazoles

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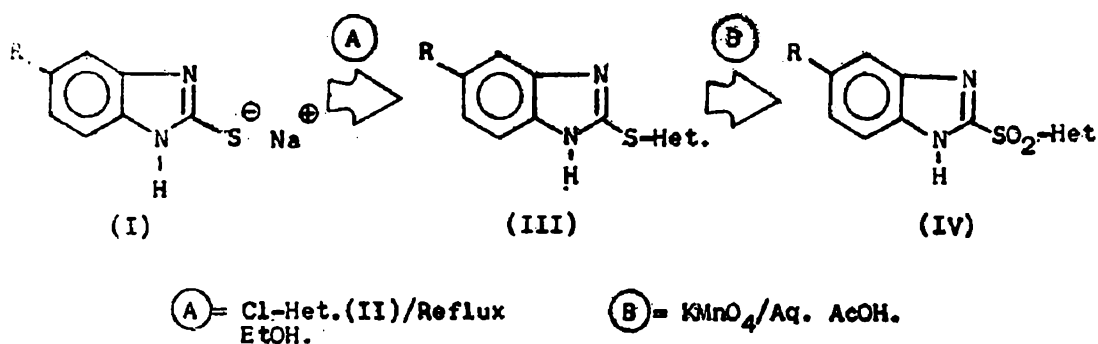
Introduction

Benzimidazole ring system have attended enormous importance in various fields of medicinal chemistry (1). Many benzimidazole derivatives have also shown antimicrobial activity (2). Thiabendazole (3) is the most important 2-heterocyclic benzimidazole derivative which acquired unique position in the field of anthelmintics as well as pesticides. Aromatic as well as heterocyclic sulfones also proved their usefulness in the antibacterial and antituberculosis field (4). These observations prompted us to prepare series

of the title compounds, with a view of testing their antituberculosis activities.

Chemistry

Sodium salt of 2-mercapto-5 (6)-substituted benzimidazole (I) was reacted with different heterocyclic halides (II) in ethanol at reflux temperature to obtain the 5(6)-substituted-2-(heterocyclic)thio, benzimidazoles (III) which were further oxidised to sulfonyl derivatives (IV) by potassium permanganate in the aqueous acetic acid by the known procedure (5).



Experimental

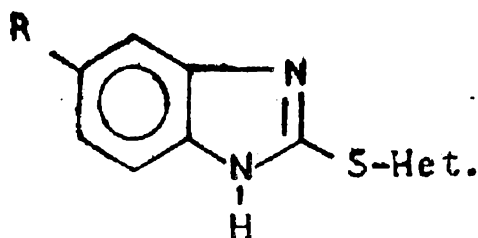
The general method for the preparation of the title compounds is illustrated by the following typical experiment:

5 (6)-Chloro-2-(2-pyrimidyl) thio, benzimidazole :

A mixture of 2.06 g of sodium salt of 5 (6)-chloro-2-mercapto benzimidazole

Table I

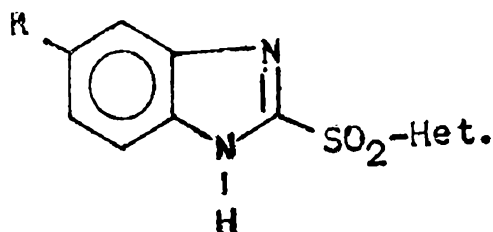
5(6)-Substituted-2-(heterocyclic)thio, benzimidazoles.



No.	Heterocycle	R	M.P.°C (uncorr.)	Mol. formula	Antituber- culosis activity (mcg/ml)
1.	2-Pyrimidyl	H	300	C ₁₁ H ₈ N ₄ S	200
2.	2-Pyrimidyl	Cl	305	C ₁₁ H ₇ ClN ₄ S	20
3.	2-Pyrimidyl	CH ₃	260	C ₁₂ H ₁₀ N ₄ S	2
1.	7-Chloro-4-quinoliny	H	130	C ₁₀ H ₁₀ ClN ₂ S	Inactive
5.	7-Chloro-4-quinoliny	Cl	146	C ₁₀ H ₁₀ Cl ₂ N ₃ S	"
6.	7-Chloro-4-quinoliny	CH ₃	130	C ₁₇ H ₁₂ ClN ₃ S	"
7.	7-Chloro-4-quinoliny	NO ₂	178	C ₁₀ H ₉ ClN ₄ O ₂ S	3.12
8.	2,6-Dichloro-4-pyrimidyl	H	202	C ₁₁ H ₆ Cl ₂ N ₄ S.HCl	12.5
9.	2,6-Dichloro-4-pyrimidyl	Cl	208	C ₁₁ H ₅ Cl ₃ N ₄ O ₃ S.HCl	12.5
10.	2,6-Dichloro-4-pyrimidyl	CH ₃	282	C ₁₂ H ₈ Cl ₂ N ₄ S	Inactive
11.	2,6-Dichloro-4-pyrimidyl	NO ₂	330	C ₁₁ H ₅ Cl ₂ N ₅ O ₂ S.HCl	50
12.	2,6-Dimethoxy-4-pyrimidyl	H	205	C ₁₃ H ₁₂ N ₄ O ₂ S	50
3.	"	Cl	79	C ₁₃ H ₁₁ ClN ₄ O ₂ S	100
14.	"	H ₃	255	C ₁₄ H ₁₄ N ₂ O ₂ S	25
5.	"	NO ₂	81	C ₁₃ H ₁₁ N ₅ O ₄ S	100

Table II

5(6)-Substituted-2-(heterocyclic)sulfonyl benzimidazoles.



No.	Heterocycle	R	M.P.°C (uncorr.)	Mol. formula	Antituber- culosis activity (mcg/ml)
1.	2-Pyrimidyl	Cl	260	C ₁₁ H ₇ ClN ₄ O ₂ S	50
2.	"	CH ₃	190	C ₁₂ H ₁₀ N ₄ O ₂ S	12.5
3.	7-Chloro-4-quinolinyl	H	175	C ₁₆ H ₁₀ ClN ₃ O ₂ S	102
4.	"	Cl	152	C ₁₆ H ₄ Cl ₂ N ₃ O ₂ S	102
5.	"	CH ₃	135	C ₁₇ H ₁₂ ClN ₃ O ₂ S	100
6.	"	NO ₂	168	C ₁₆ H ₉ ClN ₄ O ₂ S	12.5

- (a) All compounds were analysed for C, H, N and analytical results were within 0.4% of the theoretical values.
- (b) Melting points are capillary melting points and are uncorrected.
- (c) Minimum inhibitory concentration for *in vitro* antituberculosis activity (for isoniazid = 0.04 mcg/ml; and for PAS = 0.4 mcg/ml as standard
Inactive = No inhibition occur at 200 mcg/ml of *M. tuberculosis*).

(6) (0.01 mole) and 1.15 g 2-chloro-pyrimidine (0.01 mole) in 30 ml of ethanol was refluxed on steam bath for about 3 hrs. Then the solvent was removed completely under reduced pressure and some water was added, the sulphide derivative precipitated out which was collected by filtration. Recrystallised from aqueous ethanol.

Yield: 2.40 g (91.6%), m.p. 305°C.

5(6)-Chloro-2-(2-pyrimidyl)sulfonyl, benzimidazole:

2.62 g of 5(6)-chloro-2-(2-pyrimidyl), thio benzimidazole (0.01 mole) was dissolved in minimum amount of glacial acetic acid (about 20 ml) by warming on water-bath. Then 7% aqueous KMnO₄ solution was added gradually at 30-40°C. As the oxidation proceeded the pink colour of KMnO₄ solution was discharg-

ed, further KMnO_4 solution was added. This was continued till the colour of KMnO_4 solution persisted and further addition was stopped. The reaction mixture was allowed to cool to room temperature. Then NaHSO_3 was added to decompose the excess of KMnO_4 used. The reaction mixture was then poured into ice-water, where sulfonyl derivative was precipitated out and filtered, washed, with water, Recrystallised from ethanol.

Yield 1.54 gms (52%), m.p. 260°C .

Similarly, other compounds were prepared.

Biological Results

All these compounds were screened for *in vitro* antituberculosis activity against *M. tuberculosis* (H_{37}Rv strain) by the serial dilution method of Doub and Youmans (7).

The highest activity was shown upto 2 mcg/ml concentration by the compound 5(6)-methyl-2-pyrimidyl thio, benzimidazole. Compounds Nos. 2, 3, 7, 8, 9 and 14 (Table I) were active upto 25 mcg/ml concentration among the fifteen sulfide derivatives tested. Compound Nos. 2 and 6 (Table II) from sulfonyl derivatives, were active at 12 mcg/ml concentration and other compounds showed activity upto 100 mcg/ml concentration.

Acknowledgement

The authors are thankful to Dr. M. H. Shah, Assistant Director, Chemotherapy Department, Haffkine Institute, for his keen interest in the work. They are also grateful to Mr. M. T. Jaokar and his associates for elemental microanalyses. The authors are indebted to Dr. K. D. Sharma, Director, Haffkine Institute, for providing excellent facilities to carry out the present work.

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Thiamin Levels in the Brain regions during onset of Neurological Symptoms of Thiamin Deficiency in rats,

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Introduction

Thiamin deficiency has been associated with the manifestation of frank symptoms of neurological dysfunction (1). However, there has been some controversy regarding the extent of progressive reduction in brain thiamin levels of the deficient animals and simultaneous onset of biochemical and neurological aberrations (2,3). A deficit of 80% in whole brain thiamin levels has been reported in the symptomatic deficient rats (4,5). Contrary to these findings a much lesser deficit has also been recorded (6,7). Moreover, the deficiency has been implicated to result into edematous brain components (8) thereby, perhaps, distorting the expression of biochemical parameters on the basis of wet weight of the tissues.

In the course of our earlier studies on the effects of thiamin deficiency on rat brain cholinergic mechanism, we found significant reduction in brain acetylcholine levels in the symptomatic deficient rats (Gaitonde et. al., unpublished). The present studies were carried out to study the correlation between the deficits of total thiamin in the brain regions and appearance of the neurological symptoms in the deficient rats in the course of our studies. The possibility of edematous changes introducing an artifact in the expression of biochemical parameters in

the deficient animals was also checked. The results of these studies are presented here.

Materials and Methods

Weanling albino rats of Charles Foster strain from the stock of the animal house of Haffkine Biopharmaceutical Ltd. were used for the present study. Rats were matched for age, body weights and sex and were individually caged in galvanized iron cages with a wide mesh bottom so as to prevent coprophagy. The control diet contained per 100 g, 24 g defatted and devitaminized casein, 2 g vitamin mixture (9), 4 g salt mixture (10), 7 g refined ground nut oil, 20 g sucrose and 43 g potato starch. Water was provided *ad libitum*. Thiamin deficient diet contained the same vitamin mixture without thiamin. The diet, when analysed for thiamin content, was found to contain only trace amounts of the vitamin.

The rats were divided into following three groups:— (i) control diet fed *ad libitum*. (ii) thiamin deficient diet fed *ad libitum*, and (iii) control diet pair-fed to the deficient group.

Each group consisted of eight rats. The rats were sacrificed after the establishment of frank neurological symptoms (towards the end of 5th — 6th week).

The careful observations of the deficient rats revealed the following typical

Table 1
Effects of Thiamin deficiency on moisture content and Thiamin levels in Liver, Heart and Brain regions.

Group	Liver	Heart	Brain Regions		
			Cortex	Cerebellum	Medulla + Pons + Stem
Thiamin mcg per g					
1. Control, <i>ad lib</i>	8.44 ± 1.21	10.2 ± 1.7	3.02 ± 0.11	3.56 ± 0.46	5.13 ± 0.65
2. Thiamin Deficient	2.35 ^{a,b} ± 0.21	3.48 ^{a,b} ± 0.89	0.93 ^{a,b} ± 0.16	0.71 ^{a,b} ± 0.16	1.69 ^{a,b} ± 0.25
3. Control, Pair-fed	10.30 ± 1.50	12.7 ± 1.2	2.76 ± 0.31	2.41 ± 0.23	3.48 ± 0.61
Moisture Content (%)					
1. Control, <i>ad lib</i>	69.16 ± 0.09	76.40 ± 0.27	78.69 ± 0.51	77.10 ± 0.13	72.56 ± 0.60
2. Thiamin Deficient	69.72 ± 0.30	77.40 ± 1.40	77.90 ± 0.30	77.44 ± 0.79	72.22 ± 1.08
3. Control, Pair-fed.	68.44 ± 0.82	75.84 ± 0.08	78.17 ± 0.13	76.92 ± 0.76	70.59 ± 1.40

Each group consisted of eight rats. Two samples were pooled for thiamin estimation.

a, $p < 0.01$ group 2 v.s. group 1; b, $p < 0.05$ group 2 vs group 3.

symptoms progressing in sequence stated below : towards the end of second week there was some hair loss, from the third week onwards the rats developed inco-ordination in walking, impaired righting reflex followed by reluctance to walk, walking in circular motion, imbalanced locomotion and loss of righting reflex.

The different regions of the brain were separated as described by Zeman and Innes (11). Total thiamin content was estimated by the method of the Association of Official Agricultural Chemists (12). The moisture content was determined by drying the tissue at 55 °C in vacuo till a constant dry weight was obtained.

Results

As shown in Figure 1 the rats fed on control diet gained weight throughout

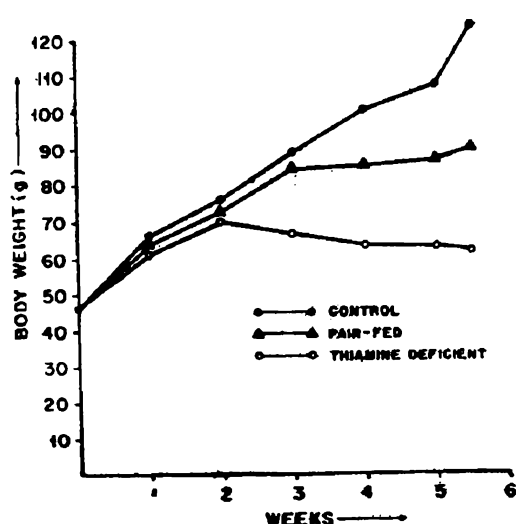


Figure — 1 :

The effects of thiamin deficiency on the growth of rats. Each point for each group of rats is the mean of eight rats. This growth pattern is typical of other groups studied.

the period of the experiment. Whereas the deficient rats showed body weight gain upto the end of second week, thereafter the weight gain declined steadily. The final body weights for the *ad lib*

control, the pair-fed control and the deficient groups were 135 ± 1.3 , 90.5 ± 9.9 and 62.3 ± 3.8 g respectively. The deficient group had a significantly reduced body weight as compared to both the controls.

The wet weights of the brain regions in the deficient group were not affected. The liver weight in the deficient group (2.62 ± 0.26 g) was significantly reduced in comparison to the *ad lib* control (5.29 ± 0.31 g). The heart weight in the deficient group (0.284 ± 0.01 g) was however significantly reduced as compared with *ad lib* (0.516 ± 0.03 g) and pair fed (0.42 ± 0.05 g) controls.

As shown in Table 1, thiamin levels were reduced in all the tissues studied. The highest deficit, as compared to the pair-fed control, was observed in liver (77%), followed by heart (72%), cerebellum (71%), cortex (66%) and medulla + pons + stem (51%).

Thiamin deficiency was found to have no effect on the moisture content of the different tissues and also of the brain regions studied (Table 1). The moisture content was highest in the cortex, followed by the cerebellum and the pooled regions.

Discussion

The profiles of body weight changes in the pair-fed controls and the deficient groups were in agreement with the reports of MaCandles et al (13). The sequence of appearance of neurological symptoms was similar to that observed by other workers (4,13). However, the deficits in thiamin level of the brain regions of the deficient animals were less (51 — 71%) as compared to the reported

values of 80% (4,5). Although 80% deficit in brain thiamin level has been generally accepted as a basal level for the onset of neurological symptoms (1), 65 — 70% deficits have been reported by Gubler (6) and much lesser deficits have been implicated by Church (8). The present studies confirm that even with lesser deficits than 80% the neurological symptoms appeared in the deficient animals. However, the extent of reduction in thiamin levels in the different regions was variable.

The moisture content of all the tissues studied remained essentially unaffected. The reported edematous changes of

astrocyte foot processes (2,3), in our opinion, require much more sensitive methods to reflect such a change and the present methodology used, in no way, can confirm these reports. However, the expression of various biochemical parameters on the basis of fresh wet weight of the tissues does not seem to involve the artifact due to gross changes in the moisture content.

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Proceedings of the Critics' Club

The activities of the Critic's Club for the period April to August 1980 began with a talk by Shri P. R. Sangole on April 18. Shri Sangole reported his work on the *in vitro* growth patterns of Langur kidney cells following *in-vivo* Hepatitis B and HIBOMA virus inoculation. HIBOMA has been demonstrated to differentially impair growth of renal cells *in-vitro*, the impairment being related to passages through which the virus has passed.

On the 2nd of May, Shri S. T. Nikam addressed the members of the Club. In his talk, he described his efforts to isolate and purify a hypoglycaemic factor from an indigenous plant. He reported the potent activity of the tubers of *Pueraria tuberosa*. Isolation of the active fraction in the form of a chemically pure substance was achieved using various chromatographic techniques on the crude methanolic extracts of the tubers. The compound responsible for hypoglycaemic activity compares favourably with standard antidiabetic agents when tested in rats.

In a guest lecture Prof. B. C. Harinath of the Mahatma Gandhi Institute of Medical Sciences, Wardha, spoke on Immunity

in filariasis. Prof. Harinath has been involved in the study of the immunological aspects of host parasite relations, his talk was based on the observation made in his laboratory recently. A liberal exchange of ideas followed the lecture, which was largely attended.

Shri M. G. Phatak of the Department of Toxicology spoke before the club on May 16. The Toxicology Department is involved in a large programme of testing commercial samples of pesticides and insecticides for their effects on laboratory animals. Shri Phatak discussed the various tests carried out on pesticides, their importance and reviewed the results obtained. Ms. Shaila Rao of the Rabies Research Unit next presented her work before the Critic's Club on the 20th of June. The worker reported the success achieved by them in establishing a cell line obtained from human embryonic tissue. The cell line has been developed with an ultimate aim of growing the rabies virus on it. In a lively discussion which followed the workers pointed out that the cell line is being studied in details for its characteristics, and attempts are being made to investigate its suitability for virus development.

Ms. Jyoti Tawakle of the Bacteriology Department discussed the development of microbes for the detoxification of industrial wastes, in talk before the club on August 22. 2, 4, Dinitro Phenol and meta Dinitro Benzene from industrial waste have been put to an indepth study. The workers have succeeded in isolating a microbe which can effectively detoxify these compounds and transform them to their less toxic metabolites. This project was undertaken with a view to develop improved waste detoxification systems.

The closing meeting of the period was addressed by Prof. J. Banatwala, Head, Clinical Virology Department, St. Thomas Hospital London. In a largely attended lecture he discussed acute liver disease in the developing countries. Prof. Banatwala discussed the role of the various viruses, viz. A, B, Non A non B, in the pathogenesis of acute liver diseases.

R. B. Ghooi
Scientific Secretary.

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Bulletin of Haffkine Institute is a triannual publication. It embodies review and research articles in biomedical sciences, mainly devoted to studies on Communicable Diseases.

Contributions should be precise and concise, highlighting original observations and inferences. The type-script (T.S.) should be sent in duplicate, typed in double space on only one side of the sheet with ample margins.

A brief summary not exceeding 200 words, should accompany the article. The title should be informative and specific, followed by author's name and address.

A short title in not more than forty letters should also be suggested.

The text of the paper should be generally sub-divided into: Introduction, Materials and Methods, or Experimental, Results, Discussion, Acknowledgement and References.

'Short Abstract' of about 100 words suitable for inclusion in 'International Abstracts of Biological Sciences', etc. should be typed on a separate sheet, with author's name, address and title of the paper.

Results should be presented concisely in tables giving only important and relevant data and avoiding incorporation of elaborate observations.

Restraint should be exercised in submitting illustrative matter. Photographs should be well-defined and on glossy paper.

References in the text should be cited by numbers serially as they appear, and to be inserted in parenthesis, e.g., Plasmid-mediated resistances to these heavy metals have also been observed in enteric bacilli, especially in P factor carrying organisms (1), and have recently been studied by several workers (2, 3, 4). References at the end should be arranged numberwise serially as they figure in the text with author's name first and abbreviations according to Index Medicus. The following pattern may be followed:

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(ii) Cruickshank, R. (1968) — Medical Microbiology, 11th Ed. 180-4, E and S Livingstone Ltd., Edinburgh, p. 28.

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