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CONTENTS

	PAGE
<i>Transmission Dynamics of Malaria : Quantitative Studies—Part IV Application of the Model for P. vivax Epidemiology</i>	.. 1
N. R. RAO, O. P. VIG, S. N. AGARWALA, S. S. SABNIS AND V. N. RAO	
<i>Synthesis and Study of 4-(p-aminophenyl) Thiazoles for in vitro Anti-tubercular Activity and as Useful Chemotherapeutic Intermediates</i>	8
D. T. CHAUDHARI, S. S. SABNIS, M. R. PATEL AND C. V. DELIWALA	
<i>Synthesis and Study of 2-arylaminopyrido (3, 2-d) Thiazoles as Possible Anthelmintic Agents</i>	16
B. G. KHADSE, M. H. SHAH, C. V. DELIWALA, M. B. BHIDE AND V. M. BHAT	
<i>Potential Anticancer Agents III : Schiff Bases derived from 4-[N, N-Bis (3- Chloropropyl) Amino] Benzaldehydes</i>	20
D. T. CHAUDHARI, S. S. SABNIS AND C. V. DELIWALA	
<i>Nutritive Value of 'Sukhadi'—A Supplementary Food</i>	.. 28
H. S. VED, V. P. SHINDE AND S. D. AMBEGAOKAR	
<i>Serological Studies in Plague</i>	33
D. M. RENAPURKAR, L. V. SARMA, SUSHILA MENON AND M. V. SANT	
<i>Fluorescent Staining for M. Tuberculosis in Tissue Sections—Comparison with Fite-Faraco Procedure</i>	37
J. K. DAVE, Y. FERNANDES AND S. C. GAWAND	
Book Review	
<i>The Blood of Sheep : Composition and Function</i>	.. 42
H. M. BHATIA	

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TRANSMISSION DYNAMICS OF MALARIA: QUANTITATIVE STUDIES—PART IV*

Application of the Model for P. vivax Epidemiology

N. R. RAO†, O. P. VIG‡, S. N. AGARWALA‡, S. S. SABNIS§ AND V. N. RAO¶

Haffkine Institute, Parel, Bombay 400 012

The epidemiological evaluation of malaria with reference to *P. vivax* infections was attempted on the basis of a sensitive mathematical model developed by the author. Input data for estimates of variables and parameters compiled from the published literature were fed into the mathematical formulae. The output data generated by computer simulation were analysed and the average duration of transmission with a set of parameters was obtained.

This study gives that when mass treatment is applied in the eradication and control of malaria, the interval between two treatments plays a greater role in the reduction of the duration of transmission. This study also gives the values for the parameters that give the lowest measure for the duration of transmission of *P. vivax* malaria.

Introduction

The epidemiological problems of 'disappearing malaria' the new phenomena peculiar to the low level transmission and the experience of the return of malaria (1, 2, 3, 4, 5) need an evaluation technique—an accurate system of measurement to determine the end-point of transmission. The system should be more sensitive and adoptable to this dynamic stage. To meet

this need, a mathematical method was developed by some of us (6) and the theory of the course of events expected were analysed (7). The computer simulation was used to reproduce a number of situations for the end of malaria transmission with a set of parameters (8).

In this paper, input data on estimates for variables and parameters of the model are described. The output data generated by computer simulation is analysed to obtain average duration of transmission with reference to *P. vivax* infections for a given set of parameters. The sensitivity of the model and the applicability of this approach for a case study of the epidemiological status of malaria in Bombay on a given data is examined.

Materials and Methods

A good account of the life cycle of the malaria parasite is known (3). We assume the knowledge of the basic facts of malaria.

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† Senior Lecturer in Epidemiology/Biostatistics, University of Dar Es Salaam, Faculty of Medicine, P. O. Box No. 20693, Dar Es Salaam, Tanzania.

‡ International Institute for Population Studies, Bombay 400 088.

§ Assistant Health Officer (Surveillance), Greater Bombay Municipal Corporation, Bombay.

¶ Director of Health Services, Government of Maharashtra, Bombay 400 001.

The incubation interval is the average time in days from ingestion of gametocytes by an anopheline to the development of infective gametocytes in a second case infected by it (9). This is considered to be eighteen days for *P. vivax* infections.

Parameter N: the time taken in days for the completion of the extrinsic cycle—According to the form of function given by Macdonald (9), the period of extrinsic development can be taken roughly as nine days for *P. vivax* applicable to tropical conditions.

Parameter P: the probability that a mosquito will survive through one day—This has received extraordinarily little study and the known efforts are very few. The probability of survival is expressed as the converse of the probability of death which is the daily mortality rate expressed as a proportion. The best series that are available to give an estimate of this parameter is from Russel *et al.* (10). From this a study, the probability of survival of *A. culicifacies* through one day is deduced as 0.775 and the expectation of life is computed as 3.8 days.

Parameter S: the sporozoite rate—Russel *et al.* (11) calculated the sporozoite rate as 0.064 per cent based on 13,145 dissections on *A. culicifacies* mosquitos. This value is considered for practical application.

Parameter A: the average number of blood meals on man taken by a mosquito in a day—This represents the man-biting index of mosquito and is a compound of the frequency of blood meals taken by the anophelis vector and of the choice of man as host. From the published literature, it is obtained that a 48-hour feeding cycle is common and that for the host preference the value as given by Russel *et al.* (11) viz. one in 40 of mosquito's feeds are on man, is considered; so that the value for the parameter 'A' would be 0.0125.

Parameter X: the proportion of bites on man which are infective to the mosquito—

This depends on the number of persons affected (whose blood contains sexual forms of malaria parasites) and the capability of the parasite or infecting agent transmitting infection. As per terminology of WHO (12) infective anophelis are female anopheles with sporozoites in their glands. As no direct measurement is available, indirect evidence is considered to arrive at the estimate. In the relationship as brought out in the expression for the sporozoite rate, when the available data were assembled to calculate the value for x , we get a numerical value for the factor ranging between 10 per cent and 17 per cent with an average of 13 per cent.

Parameter R: the recovery rate—The recovery rate is defined as the proportion of people affected (who have received one infective inoculum only) who revert to the unaffected state in element of time (9). For practical applications, the affected group will be considered as that showing parasitaemia at the time of examination and as termination is usually gradual with increasing intervals of freedom from parasitaemia (3), the recovery rate represents the increasing probability of being free from parasitaemia when examined. The only best series available to get an estimate for this variable is from Earle's work from which Macdonald (13) calculated the value for the recovery rate as 0.005. As this is a basic value which is extremely hard to collect and ethically difficult to repeat the observations from which to compute according to Colbourne (14), this value is considered as the reasonable estimate for this parameter. This rate gives that the number found positive on each successive day is the number found positive on the previous day multiplied by $(1-0.005)$.

Parameter ET: the efficacy of treatment—Treatment, here, is referred to treatment in the interruption and eradication of malaria which include various measures such as case detection, radical treatment, remedial and attack measures against the vector, etc. The proportion of people covered by all the measures in force, is considered as the numerical measure for the efficacy of treatment. Hypothetical values such as 50 per cent, 60 per cent, 70 per cent and 80 per cent are substituted in the model for this parameter to fit the observed conditions.

Parameter IBT: the interval between two treatments—Since a high proportion of the population is to be covered by various measures to free or prevent that proportion of susceptibles from the risk of malaria, with a large number of programmes operated on widely differing epidemiological backgrounds, each member of the population is covered at a more or less regular interval and therefore treatment is deemed to be at regular intervals. The period between two treatments is expressed in terms of incubation intervals. The interval between two treatments is two incubation intervals which means that more or less, the said proportion of persons in that area is covered by the protective measures at intervals of twice the period of the incubation interval of the Strain (parasite).

Variable Z_0 : the basic reproduction rate or the number of infections distributed in a community as a direct result of the process of a single primary, non-immune case—The numerical value for Z_0 is obtained by indirect means by the formula given by Macdonald (15) viz.—

$$Z_0 = \frac{p^n}{p^n - s}$$

when 'p' represents the probability of survival of a mosquito through one day, 's' represents the sporozoite rate currently

prevailing and 'n' is the period of extrinsic cycle. When the numerical values for 'n', 'p' and 's' as obtained above are substituted, we get $Z_0 = 1.005146$ for this illustration.

We now have numerical values for all the parameters and variables in the equation for the infection rate for humans, the initial rate being called the inoculation rate as per the model derived elsewhere (6) viz.—

$$h_0 \frac{AU_0}{BU_0 + C}$$

where $A = Z_0 r (v + M) = Z_0 r v$, when $M = 0$, for one season, in which Z_0 = the basic reproduction rate.

r = the recovery rate.

$V = -\log_e p$, where p is the probability that a mosquito will survive a single day; $B = ax$, where 'ax' gives the daily number of infective feeds on man i.e. the daily number of bites on man by a single mosquito which are infective to the mosquito; and $C = v + M = v$ if $M = 0$.

Results

In Part III of our series (8), we have presented the computer simulation approach to measurement of the end-point of malaria transmission, with estimates for the variables and parameters as described in the materials and method section, above.

By computer simulation, we obtained the period, in terms of incubation intervals, when malaria transmission ends due to *P. vivax* parasite. By a score of simulations we get a frequency distribution of the incubation intervals during which malaria transmission ends. We have used the values 1, 2, 3, or 4 intervals for the variable parameter IBT—interval between two treatments. Similarly we have substituted the values 80 per cent, 70 per cent, 60 per cent, 50 per cent for the variable parameter ET—efficacy of treatment. We present

below the frequency distributions with different sets of values of these varying parameters for the case of one positive and for the case of two positives.

Table I gives the frequency distribution of end-points of *P. vivax* transmission, when ET=80 per cent and when IBT=4 periods, 3 periods, 2 periods and 1 period; and for 1 positive and for 2 positives. For example, from the table it is seen that 56 per cent of times, the end-point lies in the periods of fourth to fifth incubation interval with a mean of 5.30 periods where ET=80 per cent, IBT=4 periods, and P=1 positive case. Similarly for 1

positive case, when IBT=3 periods, 70 per cent of times, the end-point lies in between fourth and fifth incubation periods, with a mean of 4.26 incubation periods for the end of malaria. Table II gives the frequency distributions with 1 positive case when the ET=70 per cent, 60 per cent and 50 per cent respectively. Tables I and II also give the mean period, the 95 per cent confidence limits for the mean and the three quartiles viz. first (25 per cent), second quartile (50 per cent) and the third quartile (75 per cent) for selected distributions. The findings are discussed in the next paragraphs.

TABLE I

*Frequency Distribution of Incubation Intervals for End of Malaria Transmission*Parasite : *P. vivax*

Length of incubation interval : eighteen days

Efficacy of treatment : 80 per cent

Number of incubation Intervals	Frequency with number of positives								
	4 intervals		3 intervals		2 intervals		1 interval		
	1+	2+	1+	2+	1+	2+	1+	2+	
0 and 1	..	7	1	4	2	6	1	2	1
2 and 3	..	12	6	2	4	40	39	48	46
4 and 5	..	56	56	35	60	3	7	..	3
6 and 7	..	5	7	8	21	1	2		..
8 and 9	..	19	19	1	1	..	..	..	..
10 and 11	..	..	1	..	8	..	1		..
12 and 13	..	1	9	..	3	..			..
14 and 15	..	..	..		1	..			..
16 and over	..	..	1		..	..			..
Total	..	100	100	50	100	50	50	50	50
Mean	..	5.30	6.69	4.26	5.40	2.90	4.31	2.01	2.40
95 per cent confidence limits	..	4.84	5.99	3.80	4.88	2.60	3.97	1.89	2.30
		5.76	7.23	4.72	5.92	3.20	4.65	2.13	2.50
Quartiles:									
Q1	..	4.01	4.30	3.18	..	3.10		1.26	
Q2	..	4.53	4.76	3.54	..	3.40		1.58	
Q3	..	5.00	8.26	3.90	..	3.77		1.89	

TABLE II

*Frequency Distribution or Incubation Intervals for End of Malaria Transmission with 1 Positive Case*Parasite : *P. vivax*

Length of incubation interval : eighteen days

No of incubation interval	Efficacy of treatment												
	70 per cent				60 per cent				50 per cent				
	IBT				IBT				IBT				
	4	3	2	1	4	3	2	1	4	3	2	1	
0 and 1	..	3	5	4	4	8	3	4	6	6	3	5	3
2 and 3	..	8	6	35	44	3	8	34	38	8	6	24	34
4 and 5	..	21	29	8	2	20	23	9	2	18	22	12	10
6 and 7	..	1	9	3	..	5	8	..	2	4	9	6	1
8 and 9	..	11	1	..	..	11	1	2	2	9	2	..	2
10 and 11	..	..	..	..	..	1	2	1	..	3	7	1	..
12 and over	..	6	..	..	..	2	5	..	..	2	1	2	..
Total	..	50	50	50	50	50	50	50	50	50	50	50	50
Mean	..	6.16	4.10	3.28	2.16	5.52							
95 per cent confidence limits.		5.10	3.58	3.24	1.99	4.64							
		7.22	4.62	3.32	2.33	6.40							
Quartiles :													
Q1	..	3.38	3.05	2.12	1.24	3.75							
Q2	..	4.60	3.50	2.53	1.60	4.66							
Q3	..	8.00	3.93	2.93	1.93								

Discussion

It is seen from the Tables I and II that the highest frequency of end of malaria is obtained in the incubation interval immediately after the treatment is applied. That is, when the interval between two treatments is 4 incubation intervals, the high frequencies of end of malaria are observed in the fifth, ninth, thirteenth and so on incubation intervals. (In the tables, the frequencies are grouped to conserve space whereas the computer output gives the frequency for each incubation interval). Similar is the case for the other values of IBT. Thus the model is sensitive depicting the parameter—the efficacy of treatment ET.

(ii) *Comparison of mean durations of transmission for different numbers of positives (*P. vivax*) within the same set of values of other parameters*—From Table I, we get the mean durations for 1 and 2 positives as 5.30 and 6.69 intervals when ET=80 per cent and IBT=4 intervals. These durations are significantly different.

When ET=80 per cent and IBT=3, the mean durations for 1 and 2 positives are 4.26 and 5.40 intervals and these are significantly different from one another.

When ET=80 per cent and IBT=2, the means are 2.90 and 4.31 which are also significantly different.

When ET=80 per cent and IBT=1, the means are 2.01 and 2.40 and these are also significantly different.

Thus it is observed that when ET = 80 per cent for all values of IBT viz .1, 2, 3 or 4 intervals, the mean duration of transmission for 1 positive and 2 positives are significantly different. But when ET = 50 per cent and IBT = 1 interval, the means for 1 and 2 positives are not significantly different (not shown in the table). Thus, the model is sensitive for small values of the variable number of positives at higher levels of treatment.

intervals are significantly different from one another; the lower the interval, the shorter the duration of transmission.

However the mean durations for the different levels of treatment are not significantly different from one another; while the durations corresponding to the different levels of intervals—particularly with reference to the first and second intervals—are significantly different. Thus the interval between treatments is more sensitive and plays a greater role than the efficacy of treatment in reducing the duration of transmission. Similar pattern

TABLE III

Mean and 95 per cent Confidence Limits of the Duration of Transmission for 1 positive by Efficacy of Treatment ET and Interval between two Treatments IBT

Parasite : *P. vivax*

Length of Interval : eighteen days

Efficacy of treatment ET	Interval between two treatments IBT											
	1			2			3			4		
50 Per cent	2.94			4.24			5.46			5.64		
	2.48	—	3.40	3.48	—	5.00	4.54	—	6.38	4.64	—	6.64
60 per cent	2.54			3.44			5.48			5.52		
	2.12	—	2.96	2.94	—	3.98	4.48	—	6.48	4.64	—	6.40
70 per cent	2.16			3.28			4.10			6.16		
	1.99	—	2.33	3.24	—	3.32	3.58	—	4.62	5.10	—	7.22
80 per cent	2.01			2.90			4.26			5.30		
	1.89	—	2.13	2.60	—	3.20	3.80	—	4.72	4.84	—	5.76

(iii) *Comparison of mean durations of transmission for different efficacies of treatment within the same set of other parameters*—The comparisons are made with reference to 1 positive (*P. vivax*) only.

Table III (summarised from Table I and II) gives the mean durations of transmission for different levels of efficacy of treatment and for different intervals between two treatments. It is seen that the mean durations given by the different

is observed with reference to quartiles (first, second and third) and is not elaborated here. The first quartile (25 per cent of the frequencies) increased from 1.26 intervals for the parameters ET=80 per cent and IBT=1 to 4.01 intervals obtained for the parameters ET=80 per cent and IBT=4. The second quartile (50 per cent of the frequencies) ranges from 1.58 intervals of parameters ET=80 per cent and IBT=1 to 4.66 intervals of parameters ET=60

per cent and IBT=4. The third quartile (75 per cent of the frequencies) ranges from 1.89 intervals of parameters ET=80 per cent and IBT=1 to 8 intervals of parameters ET=70 per cent and IBT=4. Thus the parameters ET=80 per cent and IBT=1 interval gives the lowest measure for the duration of transmission (Mean as well as the first, second and third quartiles). It is, therefore, postulated that the interval between two treatments, plays a greater role in the duration of transmission of malaria and that, the lower the interval between the two treatments, the shorter the duration of transmission or the quicker the end of *P. vivax* malaria transmission.

This study gives the lowest mean duration of transmission with ET=80 per cent and IBT=1 interval and for 1 positive as 2.01 intervals for *P. vivax* infections equivalent to thirty-six days. This could be the basis for the demarcation of interrupted zones with the detection of a *P. vivax* positive case in the zone or sector during an equivalent period. That is, on the basis that if no positive *P. vivax* case is detected in a sector for a period of thirty-six days, then the transmission is deemed to be interrupted. In the next part of the series (16) when the epidemiological evaluation with reference to *P. falciparum* is attempted, the full status of malaria can be visualised.

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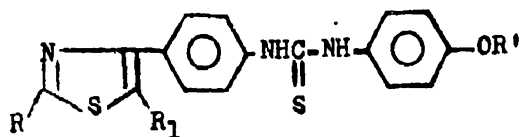
SYNTHESIS AND STUDY OF 4-(p-AMINOPHENYL) THIAZOLES FOR IN VITRO ANTITUBERCULAR ACTIVITY AND AS USEFUL CHEMOTHERAPEUTIC INTERMEDIATES

D. T. CHAUDHARI, S. S. SABNIS, M. R. PATEL AND C. V. DELIWALA
Department of Chemotherapy, Haffkine Institute, Bombay 400 012

In view of the broad spectrum of antibacterial and anthelmintic activity displayed by thiazole derivatives, various new 4-(p-aminophenyl) thiazoles have been prepared. In all sixty-three thiazoles have been synthesized and screened for antitubercular activity. Most of these showed good antitubercular activity. Further, these thiazoles have been used as intermediates to prepare other chemotherapeutically useful compounds.

Introduction

High degree of antitubercular activity has been reported from this laboratory for thio-carbanilides (I) obtained from 4-(p-aminophenyl) thiazoles and alkoxyphenylisothiocyanates (1).



(I)

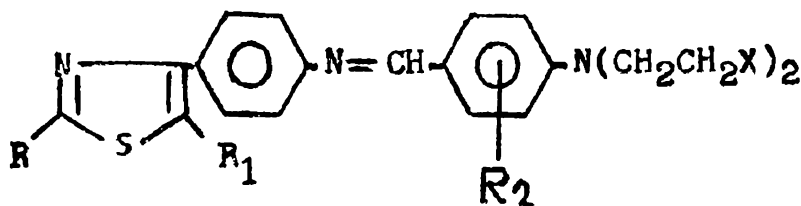
Recently, a number of Schiff bases II and III containing thiazole moiety have shown considerable amount of activity in experimental tumor systems and some have also recorded 'cures' in Dunning

Leukemia, as well as in L-1210 lymphoid and P 388 lymphocytic leukemia (2,3,4).

In view of the above, we have now prepared the series of new 4-(p-aminophenyl) thiazoles (VI) having aryloxy-methyl, aralkyl or alkyl substituents in 2-position and methyl group in 5-position for the purpose of incorporating them into new molecules to see the change in biological activity.

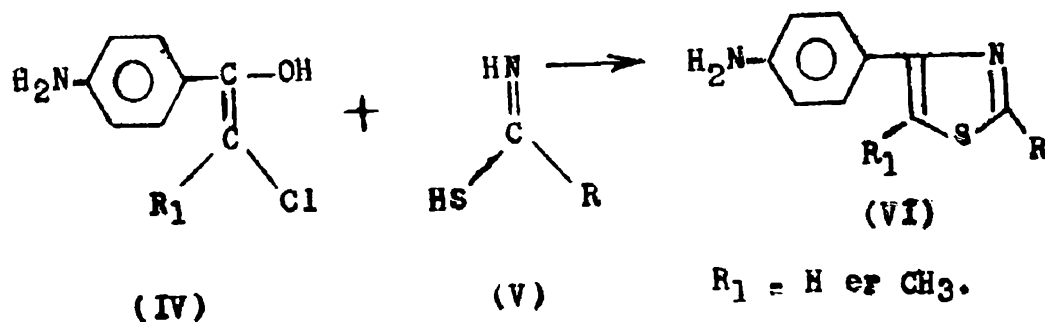
Chemistry

The synthesis of 4-(p-aminophenyl) thiazoles was followed by reacting equimolecular mixture of p-aminophenyl halo-ketones (IV) and various thioamides (V) and isolating the hydrochlorides of thiazoles (VI).



II : X = -Cl

III : X = $\text{-O-S(=O)}_2\text{-CH}_3$



2-Chloro-4'-aminoacetophenone (IV— $R_1 = \text{H}$) and 2-chloro-4'-aminopropiophenone (IV— $R_1 = \text{CH}_3$) were obtained in 60-70 per cent yield by treatment of acetanilide with chloroacetyl chloride and ω -chloropropionylchloride respectively in presence of anhydrous aluminium chloride under conditions of Friedel-Craft reaction followed by hydrolysis (1).

Thioamides (V) required to synthesize the thiazoles were prepared by the treatment of appropriate amides with phosphorous pentasulphide (5). Most of the thiobenzamides were prepared using basic catalyst like pyridine and triethylamine along with nitriles, and passing hydrogen sulphide gas at room temperature and at atmospheric pressure (6). It was, however, observed that when the benzonitriles were associated with phenolic hydroxy or carboxy group, the yields of thioamides were very poor. In such cases, the thioamides were obtained in good yields by heating the corresponding nitriles and hydrogen sulphide under pressure in closed vessel with basic catalyst like triethylamine pyridine and ammonia. The nitriles required for the preparation of thioamides were prepared by customary methods (7-10).

Experimental

3-Ethoxy-4-hydroxythiobenzamide—A mixture of 3-ethoxy-4-hydroxybenzonitrile (8.2 g, 0.05 mole) in ethanol (25 ml), saturated with ammonia and containing triethylamine (12 ml) was placed in a steel

bomb and cooled in ice-bath. The mixture was saturated by bubbling dry hydrogen sulphide and then the closed bomb was heated in steam-bath for two hours. After cooling to 0°C in ice, it was opened and the mixture poured in an evaporating dish. The solvent was evaporated at room temperature and the viscous mass that remained behind was taken in 150 ml water, acidified with dilute hydrochloric acid to pH 4. It was extracted with ether (100 ml $\times$ 2). The ethereal solution dried over anhydrous sodium sulphate and the ether removed. The solid compound that was obtained, was recrystallised from benzene to give pale yellow needles. Yield 8.1 g (82 per cent), m.p. $145-146^\circ\text{C}$.

3-Ethoxy-4-benzyloxythiobenzamide—3-Ethoxy-4-benzyloxybenzonitrile (12.65 g, 0.05 mole) was dissolved in pyridine (20 ml) and triethylamine (10 ml) in 250 ml conical flask, and dry hydrogen sulphide gas was bubbled through this mixture for a period of six hours. The mixture was then poured in 250 ml of ice-water. The precipitated 4-benzyloxy-3-ethoxythiobenzamide was filtered on buchner funnel with suction and air dried. It was recrystallised from benzene to shining yellow needles. Yield 10.9 g (76 per cent), m.p. $140-141^\circ\text{C}$.

2-(p-Chlorothymoxymethyl)-4-(p-amino-phenyl)thiazole monohydrochloride—A mixture of p-chlorothymoxythioacetamide (2.85 g, 0.011 mole), ω -chloro-p-aminoacetophenone (1.7 g, 0.01 mole) and

absolute ethanol (8 ml) was refluxed. The solid thiazole hydrochloride separated while the mixture was refluxing. The mixture was filtered while hot, and the solid washed with ether to yield the titled thiazole hydrochloride 2.9 g (71 per cent). The compound was of analytical purity, m.p. 237–240°C.

Biological results

The *in vitro* tuberculostatic activity was determined on culture of *M. tuberculosis* Var. *Homini* (Strain H₃₇ Rv) cultivated in Youman's medium containing 10 per cent horse serum by a modified method of Doub and Youman (11) and previously reported from this laboratory (12). Out of sixty-three thiazoles, forty-two representative members were tested and majority of these had *in vitro* tuberculostatic activity

below 10 µg/ml. Compound number 15 and 38 showed activity at 0.2 µg/ml. Compounds 20, 30, 37 and 40 had minimum inhibitory concentration of 0.5 µg/ml. The thiazoles having methyl group at 5-position showed higher activity than the corresponding thiazoles devoid of methyl group at 5-position.

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TABLE I
Benzonitriles
R-CN

No.	R	Yield per cent	b.p./m.p. °C (Uncorr.) ^a	Molecular formula*
1	p-Propoxyphenyl	90	46–00	C ₁₀ H ₁₁ NO
2	3-Methoxy-4-propoxyphenyl	76	82–83	C ₁₂ H ₁₃ NO ₂
3	3-Methoxy-4-benzyloxyphenyl	81	92–93	C ₁₅ H ₁₃ NO ₂
4	3-Ethoxy-4-methoxyphenyl	82	71–72	C ₁₀ H ₁₁ NO ₂
5	3, 4-Diethoxyphenyl	84	70–71	C ₁₁ H ₁₃ NO ₂
6	3-Ethoxy-4-propoxyphenyl	78	55–56	C ₁₂ H ₁₅ NO ₂
7	3-Ethoxy-4-benzyloxyphenyl	85	65–66	C ₁₆ H ₁₅ NO ₂

TABLE II
Aryloxyacetoneitriles
R-OCH₂ CN

No.	R	Yield per cent	b.p./m.p. °C (uncorr.) ^a	Molecular formula*
8	p-Bromophenyl	75	145–148/5 mm	C ₈ H ₆ BrNO
9	p-Acetamidophenyl	78	135–136	C ₁₀ H ₁₀ N ₂ O ₂
10	2, 4-Dibromophenyl	82	77–78	C ₈ H ₆ Br ₂ NO
11	o-Methoxyphenyl	64	138–140/5 mm	C ₉ H ₉ NO ₂
12	2-iso-Propyl-4-chloro-3-methylphenyl (p-Chlorothymoxyacetoneitrile)	81	62–63	C ₁₂ H ₁₄ NOCl
13	2-Bromo-4-methylphenyl	80	58–59	C ₉ H ₈ BrNO
14	2, 6-Dibromo-4-methylphenyl	76	112–113	C ₉ H ₇ Br ₂ NO

TABLE III

Thioamides

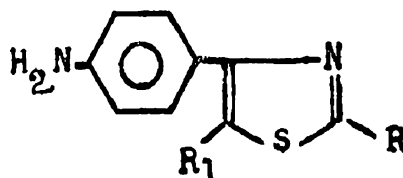
No.	R	Yield per cent	m.p. °C (uncorr.) ^a	Molecular formula*
1	p-Bromophenyl ..	93	144-00	C ₇ H ₆ BrNS
2	m-Bromophenyl ..	76	105-00	C ₇ H ₆ BrNS
3	o-Bromophenyl ..	78	80-81	C ₇ H ₆ BrNS
4	o-Tolyl ..	68	88-00	C ₈ H ₉ NS
5	p-Hydroxyphenyl ..	48	200-202 ^b	C ₇ H ₇ NOS
6	p-Ethoxyphenyl ..	93	162-163	C ₉ H ₁₁ NOS
7	p-β-Hydroxyethoxyphenyl ..	90	134-135	C ₉ H ₁₁ NO ₂ S
8	p-Propoxyphenyl ..	95	144-145	C ₁₀ H ₁₃ NOS
9	3-Ethoxy-4-hydroxyphenyl ..	82	145-146	C ₉ H ₁₁ NO ₂ S
10	3-Methoxy-4-ethoxyphenyl ..	86	155-156	C ₁₀ H ₁₃ NO ₂ S
11	3-Methoxy-4-propoxyphenyl ..	88	128-129	C ₁₁ H ₁₅ NO ₂ S
12	3-Methoxy-4-benzyloxyphenyl ..	92	165-166	C ₁₅ H ₁₅ NO ₂ S
13	3-Ethoxy-4-methoxyphenyl ..	92	149-150	C ₁₀ H ₁₃ NO ₂ S
14	3, 4-Diethoxyphenyl ..	84	157-158	C ₁₁ H ₁₅ NO ₂ S
15	3-Ethoxy-4-benzyloxyphenyl ..	76	140-141	C ₁₆ H ₁₇ NO ₂ S
16	3-Ethoxy-4-propoxyphenyl ..	78	132-133	C ₁₂ H ₁₇ NO ₂ S
17	p-Methoxybenzyl ..	91	143-144	C ₉ H ₁₁ NOS

TABLE IV

Aryloxythioacetamide

No.	R	Yield per cent	m.p. °C (uncorr.) ^a	Molecular formula*
18	m-Toluoxy ..	83	95-96	C ₉ H ₁₁ NOS
19	p-Bromophenoxy ..	74	131-132	C ₈ H ₈ BrNOS
20	2, 4-Dichlorophenoxy ..	78	138-140	C ₈ H ₇ Cl ₂ NOS
21	2, 4-Dibromophenoxy ..	75	175-176	C ₈ H ₇ Br ₂ NOS
22	p-Chloro-m-creasoy ..	79	139-140	C ₉ H ₁₀ ClNOS
23	p-Chlorothymoxy ..	75	168-170	C ₁₂ H ₁₆ ClNOS
24	2-Bromo-4-Methylphenoxy ..	78	168-169	C ₉ H ₁₀ BrNOS
25	2, 6-Dibromo-4-methylphenoxy ..	71	158-159	C ₉ H ₉ Br ₂ NOS
26	p-Acetamidophenoxy ..	82	175-176	C ₁₀ H ₁₂ N ₂ O ₂ S
27	Guicoxy ..	64	89-90	C ₉ H ₁₁ NO ₂ S
28	β-Naphthoxy ..	90	168-169	C ₁₂ H ₁₁ NOS

TABLE V
4-(*p*-Aminophenyl) Thiazoles



No	R	R ₁	Yield %	m.p. °C (uncorr.) ^a	Molecular formula*	Antitubercular activity**
1	<i>p</i> -ClC ₆ H ₄	H	82	277-280	C ₁₅ H ₁₁ ClN ₂ S.HCl	200
2	<i>p</i> -ClC ₆ H ₄	CH ₃	72	271-272	C ₁₆ H ₁₃ ClN ₂ S.HCl	2
3	<i>m</i> -ClC ₆ H ₄	CH ₃	70	260-263	C ₁₆ H ₁₃ ClN ₂ S.HCl	10
4	<i>o</i> -ClC ₆ H ₄	H	79	252-255	C ₁₅ H ₁₁ ClN ₂ S.HCl	—
5	<i>o</i> -ClC ₆ H ₄	CH ₃	62	271-274	C ₁₆ H ₁₃ ClN ₂ S.HCl	—
6	<i>p</i> -BrC ₆ H ₄	H	83	288-290	C ₁₅ H ₁₁ BrN ₂ S.HCl	200
7	<i>p</i> -BrC ₆ H ₄	CH ₃	75	275-278	C ₁₆ H ₁₃ BrN ₂ S.HCl	10
8	<i>m</i> -BrC ₆ H ₄	H	78	265-268	C ₁₅ H ₁₁ BrN ₂ S.HCl	—
9	<i>m</i> -BrC ₆ H ₄	CH ₃	66	268-271	C ₁₆ H ₁₃ BrN ₂ S.HCl	10
10	<i>o</i> -BrC ₆ H ₄	H	82	258-261	C ₁₅ H ₁₁ BrN ₂ S.HCl	—
11	<i>o</i> -BrC ₆ H ₄	CH ₃	64	272-275	C ₁₆ H ₁₃ BrN ₂ S.HCl	—
12	<i>o</i> -HOC ₆ H ₄	H	74	278-281	C ₁₅ H ₁₂ N ₂ OS.HCl	2
13	<i>o</i> -HOC ₆ H ₄	CH ₃	48	282-285	C ₁₆ H ₁₄ N ₂ OS.HCl	10
14	<i>p</i> -HOC ₆ H ₄	H	80	280-283	C ₁₅ H ₁₂ N ₂ OS.HCl	2
15	<i>p</i> -HOC ₆ H ₄	CH ₃	63	258-262	C ₁₆ H ₁₄ N ₂ OS.HCl	0.2
16	3,4-EtO(HO)C ₆ H ₃	H	83	232-235	C ₁₇ H ₁₆ N ₂ O ₂ S.HCl	1
17	3,4-MeO(HO)C ₆ H ₃	H	81	262-263	C ₁₆ H ₁₄ N ₂ O ₂ S.HCl	5
18	3,4-EtO(HO)C ₆ H ₃	CH ₃	65	244-247	C ₁₈ H ₁₈ N ₂ O ₂ S.HCl	1
19	3,4-MeO(HO)C ₆ H ₃	CH ₃	70	242-245	C ₁₇ H ₁₆ N ₂ O ₂ S.HCl	1
20	<i>p</i> -EtOC ₆ H ₄	H	82	250-253	C ₁₇ H ₁₆ N ₂ OS.HCl	0.5
21	<i>p</i> -EtOC ₆ H ₄	CH ₃	78	275-278	C ₁₈ H ₁₈ N ₂ OS.HCl	1
22	<i>p</i> -HOCH ₂ CH ₂ OC ₆ H ₄	H	74	232-235	C ₁₇ H ₁₆ N ₂ O ₂ S.HCl	2
23	<i>p</i> -HOCH ₂ CH ₂ OC ₆ H ₄	CH ₃	51	244-248	C ₁₈ H ₁₈ N ₂ O ₂ S.HCl	—

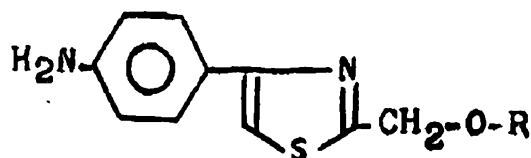
TABLE V—contd.

No.	R	R ₁	Yield %	m.p. °C (uncorr.) ^a	Molecular formula [*]	Antituber- cular Activity ^{**}
24	p-(n)PrOC ₆ H ₄	H	82	245-248	C ₁₈ H ₁₈ N ₂ O S.HCl	2
25	p-(n)PrOC ₆ H ₄	CH ₃	54	277-280	C ₁₉ H ₂₀ N ₂ OS.HCl	1
26	3,4-(MeO) ₂ C ₆ H ₃	H	78	248-252	C ₁₇ H ₁₆ N ₂ O ₂ S.HCl	-
27	3,4-(EtO) ₂ C ₆ H ₃	H	74	225-229	C ₁₉ H ₂₀ N ₂ O ₂ S.HCl	2
28	3,4-(EtO) ₂ C ₆ H ₃	CH ₃	61	234-237	C ₂₀ H ₂₂ N ₂ O ₂ S.HCl	1
29	3,4-(EtO)(MeO)C ₆ H ₃	H	79	221-224	C ₁₈ H ₁₈ N ₂ O ₂ S.HCl	1
30	3,4-EtO(MeO)C ₆ H ₃	CH ₃	65	255-258	C ₁₉ H ₂₀ N ₂ O ₂ S.HCl	0.5
31	3,4-EtO(PrO)C ₆ H ₃	H	75	214-217	C ₂₀ H ₂₂ N ₂ O ₂ S.HCl	2
32	3,4-EtO(PrO)C ₆ H ₃	CH ₃	68	217-220	C ₂₁ H ₂₄ N ₂ O ₂ S.HCl	20
33	3,4-EtO(PhCH ₂ O)C ₆ H ₃	H	80	200-203	C ₂₄ H ₂₂ N ₂ O ₂ S.HCl	-
34	3,4-EtO(PhCH ₂ O)C ₆ H ₃	CH ₃	64	215-218	C ₂₅ H ₂₄ N ₂ O ₂ S.HCl	2
35	3,4-MeO(EtO)C ₆ H ₃	H	78	215-218	C ₁₈ H ₁₈ N ₂ O ₂ S.HCl	-
36	3,4-MeO(EtO)C ₆ H ₃	CH ₃	67	243-246	C ₁₉ H ₂₀ N ₂ O ₂ S.HCl	-
37	3,4-MeO(PrO)C ₆ H ₃	H	76	207-210	C ₁₉ H ₂₀ N ₂ O ₂ S.HCl	0.5
38	3,4-MeO(PrO)C ₆ H ₃	CH ₃	68	220-223	C ₂₀ H ₂₂ N ₂ O ₂ S.HCl	0.2
39	3,4-MeO(PhCH ₂ O)C ₆ H ₃	H	83	219-222	C ₂₃ H ₂₀ N ₂ O ₂ S.HCl	100
40	3,4-MeO(PhCH ₂ O)C ₆ H ₃	CH ₃	60	215-218	C ₂₄ H ₂₂ N ₂ O ₂ S.HCl	0.5
41	p-CH ₃ CONHC ₆ H ₄	CH ₃	60	285-288	C ₁₈ H ₁₇ N ₃ OS.HCl	1
42	3,4,5-(MeO) ₃ C ₆ H ₂	H	80	242-245	C ₁₈ H ₁₈ N ₂ O ₃ S.HCl	2
43	3,4,5-(MeO) ₃ C ₆ H ₂	CH ₃	62	242-245	C ₁₉ H ₂₀ N ₂ O ₃ S.HCl	2
44	2,4-Cl ₂ C ₆ H ₃	H	75	264-267	C ₁₅ H ₁₀ Cl ₂ N ₂ S.HCl	-
45	2,4-Cl ₂ C ₆ H ₃	CH ₃	51	295-298	C ₁₆ H ₁₂ Cl ₂ N ₂ S.HCl	-
46	Benzyl	CH ₃	51	2207-210	C ₁₇ H ₁₆ N ₂ S.HCl	1
47	p-MeOC ₆ H ₄ CH ₂	CH ₃	59	177-180	C ₁₈ H ₁₈ N ₂ OS.HCl	20
48	p-ClC ₆ H ₄ CH ₂	CH ₃	66	235-238	C ₁₇ H ₁₅ ClN ₂ S.HCl	-
49	p-BrC ₆ H ₄ CH ₂	H	79	221-224	C ₁₆ H ₁₃ BrN ₂ S.HCl	-
50	CH ₃	CH ₃	80	278-280	C ₁₁ H ₁₂ N ₂ S.2HCl	10

TABLE V—contd.

Sr. No.	R	R ₁	Yield %	M.p. °C (uncorr.) ^a	Molecular formula*	Antitubercular Activity** (μg/ml)
51	H	H	72	99-100 ^c	C ₉ H ₈ N ₂ S (Base)	200
52	CH ₃	H	74	145-146 ^d	C ₁₀ H ₁₀ N ₂ S (Base)	200
53	<i>p</i> -CH ₃ C ₆ H ₄	CH ₃	66	263-265	C ₁₇ H ₁₆ N ₂ S.HCl	-
54	<i>m</i> -CH ₃ C ₆ H ₄	H	75	252-255	C ₁₆ H ₁₄ N ₂ S.HCl	-
55	<i>m</i> -CH ₃ C ₆ H ₄	CH ₃	60	250-253	C ₁₇ H ₁₆ N ₂ S.HCl	-
56	<i>o</i> -CH ₃ C ₆ H ₄	H	74	254-258	C ₁₆ H ₁₄ N ₂ S.HCl	-

TABLE VI

2-Aryloxymethyl-4-(*p*-aminophenyl) Thiazoles

Sr. No.	R	Yield %	M.p. °C (uncorr.) ^a	Molecular formula*	Antitubercular activity** (μg/ml)
57	<i>m</i> -CH ₃ C ₆ H ₄	78	222-225	C ₁₇ H ₁₆ N ₂ O S.HCl	-
58	<i>p</i> -CH ₃ CONHC ₆ H ₄	84	248-251	C ₁₈ H ₁₇ N ₃ O ₂ S.HCl	10
59	<i>p</i> -ClC ₆ H ₄	85	242-245	C ₁₆ H ₁₃ ClN ₂ OS.HCl	20
60	2,4,5- <i>iso</i> -C ₃ H ₇ , CH ₃ , ClC ₆ H ₂	71	237-240	C ₂₀ H ₂₁ ClN ₂ OS.HCl	20
61	2,6-Br ₂ , 4-CH ₃ C ₆ H ₂	84	245-246	C ₁₇ H ₁₆ Br ₂ N ₂ OS.HCl	-
62	2,4-Br, CH ₃ C ₆ H ₃	81	205-207	C ₁₇ H ₁₅ BrN ₂ OS.HCl	-
63	<i>β</i> -Naphthyl	75	232-235	C ₂₀ H ₁₆ N ₂ OS.HCl	-

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

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(d) Please see Ref. No. 1.

(*) Analysed correctly for C, H, and N ± 0.4 %.

(**) Minimum Inhibitory Concentration *in vitro* Antitubercular Action μg/ml (For Isoniazid 0.04 μg/ml, For PAS=0.4/μg/ml).

(-) Compounds not tested.

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SYNTHESIS AND STUDY OF 2-ARYLAMINOPYRIDO (3, 2-d) THIAZOLES AS POSSIBLE ANTHELMINTIC AGENTS

B. G. KHADSE, M. H. SHAH, C. V. DELIWALA, M. B. BHIDE AND V. M. BHAT

Department of Chemotherapy, Haffkine Institute, Parel, Bombay 400 012

New 2-arylamino-pyrido (3, 2-d) thiazoles are prepared by the reaction of arylamines with 2-chloro-3-isothiocyanatopyridine or of various aryl isothiocyanates with 2-chloro-3-aminopyridine. Many of them have manifested promising activity when tested against *N. dubius* in mice.

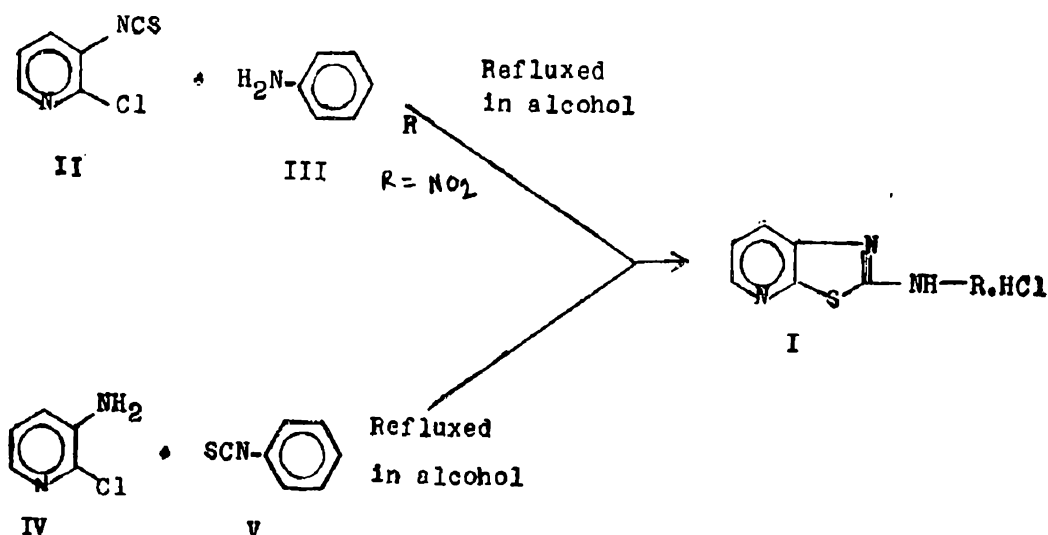
In continuation of our work on the synthesis of pyrido (3, 2-d) thiazole derivatives (I), it was considered of interest to exploit the 2-position of this molecule further to obtain the 2-aryl-substituted pyrido (3, 2-d) thiazoles with a view to test them as anthelmintic agents.

Chemistry

The desired 2-arylamino-pyrido (3, 2-d) thiazoles could be prepared by reacting 2-chloro-3-isothiocyanatopyridine (II) and

various arylamines (III) with simultaneous condensation and cyclization.

Alternatively, 2-chloro-3-aminopyridine (IV) also reacted with arylisothiocyanates (V) to give the same compound (I). While a number of compounds could be prepared by both these methods, the arylamines with nitro group (III) did not react with 2-chloro-3-isothiocyanatopyridine. Such compounds, however, could easily be obtained in excellent yields by using nitroarylisothiocyanates to react with 2-chloro-3-aminopyridine.



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An ethereal linkage para to the amino group in phenoxyalkane series is essential for potential anthelmintic activity (2). Bithinol, an important fasciolicide used in veterinary medicine contains halogenated phenol as the main molecule (3,4). Similarly, the presence of a ketone linkage either in cyclic or in open chain structure seems responsible for anthelmintic activity (5). These features are observable in drugs like santonin, mebendazole (6) or parbendazole (7). These facts guided us to synthesize the pyridothiazole derivatives incorporating each of the groupings mentioned above.

Experimental

2-[(*p*-Methoxyphenyl) amino] pyrido (3, 2-*d*) thiazole hydrochloride—In a 100 ml round bottomed flask was taken 2-chloro-3-aminopyridine (1.28 g, 0.01 mole) in ethanol (25 ml). To it was added *p*-methoxyphenyl isothiocyanate (1.65 g, 0.01 mole). The reaction mixture was refluxed for six hours and the crystalline compound obtained was collected by filtration. It was recrystallised from ethanol. Yield 86 per cent (2.56 g), m.p. 253–54°.

Analysis calculated for $C_{13}H_{11}N_3OS \cdot HCl$: C 53.24; H 4.09; N 14.33 per cent. Found C 53.16; H 3.78; N 14.52 per cent.

2-[(*p*-Nitrophenyl) amino] pyrido (3, 2-*d*) thiazole hydrochloride—In 100 ml round bottomed flask were taken 2-chloro-3-aminopyridine (1.28 g, 0.01 mole) and ethanol (25 ml). To this was added *p*-nitrophenyl isothiocyanate (1.8 g, 0.01 mole) and the reaction mixture refluxed for seven hours. The voluminous pale yellow compound obtained was collected by filtration. It was recrystallized from ethanol. Yield 80 per cent (2.5 g), m.p. 300°.

Analysis calculated for $C_{12}H_8N_4O_2S \cdot HCl$: C 45.75; H 2.85; N 18.19 per cent. Found C 46.45; H 2.53; N 17.45 per cent.

Biological screening results

Most of the compounds were tested against *N. dubius* in mice. L-Tetramisole was taken as the standard drug, which showed 100 per cent mortality of the adult worm at the dose level of 0.1 mg. The activity shown by the compounds tested is given in Table I.

Discussion

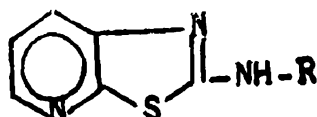
Almost all the compounds were tested against *N. dubius* in mice for their anthelmintic activity. One of the compounds, namely, 2-(*o*-methoxyphenyl) aminopyrido (3, 2-*d*) thiazole showed activity causing 77 per cent mortality of the adult worms at the single dose of 0.1 mg and at 0.5 mg dose it was 91 per cent. When the methoxy group was shifted to the para position the activity was reduced. Similarly, the 3, 4-dimethoxy or 3, 4-5-trimethoxyphenyl analogues did not show good activity. The increase in length of the carbon chain at *p*-position in the alkoxy derivatives of the pyridothiazole made no significant change in the activity.

The compounds with chlorine atom at para position in the phenyl nucleus, at 0.5 mg dose showed the mortality rate upto 70 per cent; however, 3, 4-dichlorophenyl derivative reduced it. The compounds with the nitro group at *o* or *p*-position have caused the same mortality rate i.e., upto the extent of 87 per cent at 0.5 mg dose level. The benzoyl and halobenzoyl derivatives were equally active.

Acknowledgements

The authors are indebted to Mr. S. S. Mahajani, Department of Pharmacology, for making the screening data available, and to Mr. M. T. Jaokar and his associates for microanalyses. Thanks are also due to the Director, Haffkine Institute, Bombay for his interest and providing facilities to carry out the present work.

TABLE I
2-Arylaminoipyrido (3, 2-d) Thiazoles)



S.No.	R	M.p. °C (uncorr.) ^a	Yield %	Molecular formula ^b	Dose in mg	% Mortality of adult worms after 7 days of the drug.
1.	Phenyl	273-74	70	C ₁₂ H ₉ N ₃ S.HCl	0.1 0.5	40 60
2.	Benzyl	229-30	60	C ₁₃ H ₁₁ N ₃ S.HCl		
3.	Phenylethyl	220-21	60	C ₁₄ H ₁₃ N ₃ S.HCl		
4.	m-Tolyl	243-44	60	C ₁₃ H ₁₁ N ₃ S.HCl		
5.	p-Tolyl	198-200	62	C ₁₃ H ₁₁ N ₃ S.HCl	0.1 0.5	72 85
6.	p-Hydroxyphenyl	255-57	50	C ₁₂ H ₉ N ₃ OS.HCl	0.1 0.5	38 83
7.	m-Hydroxyphenyl	270-72	50	C ₁₂ H ₉ N ₃ OS.HCl		
8.	o-Hydroxyphenyl	255-57	50	C ₁₂ H ₉ N ₃ OS.HCl		
9.	p-Methoxyphenyl	253-54	70	C ₁₃ H ₁₁ N ₃ OS.HCl	0.1 0.5	30 55
10.	p-Ethoxyphenyl	237-38	65	C ₁₄ H ₁₃ N ₃ OS.HCl		
11.	p-n-Propoxyphenyl	225-27	55	C ₁₅ H ₁₅ N ₃ OS.HCl	0.1 0.5	70 87
12.	p-n-Butoxyphenyl	222-224	50	C ₁₆ H ₁₇ N ₃ OS.HCl	0.1 0.5	70 87
13.	p-Chlorophenyl	255-256	65	C ₁₂ H ₈ ClN ₃ S.HCl	0.1 0.5	45 70
14.	m-Chlorophenyl	258-260	65	C ₁₂ H ₈ ClN ₃ S.HCl		
15.	2,6-Dichlorophenyl	213-214	50	C ₁₂ H ₇ Cl ₂ N ₃ S.(F.B)		
16.	3,4-Dichlorophenyl	273-274	60	C ₁₂ H ₇ Cl ₂ N ₃ S.(F.B)	0.1 0.5	25 60
17.	2,6-Dichlorophenyl ^b	179-180	50	C ₁₂ H ₇ Cl ₂ N ₃ S.HCl		
18.	3,5-Dichloro-4-hydroxyphenyl	284-85	50	C ₁₂ H ₇ Cl ₂ N ₃ OS.HCl	0.1 0.5	63 88
19.	p-Chloroacetylphenyl	272-274	60	C ₁₄ H ₁₀ ClN ₃ OS.HCl		
20.	2-Chloro-5-nitrophenyl	262-63	60	C ₁₂ H ₇ ClN ₄ O ₂ S.HCl	0.1 0.5	50 62
21.	o-Nitrophenyl	164-165	80	C ₁₂ H ₈ N ₄ O ₂ S.HCl	0.1 0.5	50 75
22.	p-Nitrophenyl	298-300	80	C ₁₂ H ₈ N ₄ O ₂ S.HCl	0.1 0.5	
23.	o-(2'-Carboxymethoxy-4'-nitro-phenyl)	148-150	55	C ₁₄ H ₁₀ N ₄ O ₄ S.HCl		
24.	2-Hydroxy-4-carboxyphenyl	102-104	45	C ₁₃ H ₉ N ₃ O ₃ S.HCl		
25.	2,5-Dimethoxyphenyl	115-117	40	C ₁₄ H ₁₀ N ₃ O ₂ S.HCl		
26.	3,4-Dimethoxyphenyl	236-238	55	C ₁₄ H ₁₃ N ₃ O ₂ S.HCl		
27.	3,4,5-Trimethoxyphenyl	232-234	60	C ₁₆ H ₁₅ N ₃ O ₃ S.HCl	0.1 0.5	25 60
28.	2-Benzoylphenyl	149-160	74	C ₁₉ H ₁₃ N ₃ OS.HCl (F.B.)	0.1 0.5	74 80
29.	2-Benzoyl-4-chlorophenyl	268-270	70	C ₁₉ H ₁₂ N ₃ OS.HCl	0.1 0.5	82 88
b-Tetramisole					0.1 0.5	100

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

(b) - Literature m.p. 179-80°C. F.B. = Free base.

* - Analyzed correctly for C, H, N within ± 0.4%.

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POTENTIAL ANTICANCER AGENTS III: SCHIFF BASES DERIVED FROM 4-[N,N-BIS (3-CHLOROPROPYL) AMINO] BENZALDEHYDES

D. T. CHAUDHARI, S. S. SABNIS AND C. V. DELIWALA

Department of Chemotherapy, Haffkine Institute, Parel, Bombay 400 012

Schiff bases from 4-[N, N-bis (3-chloropropyl)-amino] benzaldehydes having methyl, chloro, bromo or methoxy groups at 2 or 3 position and various aromatic amines have been synthesized and screened for antitumor activity against L-1210 lymphoid leukemia. None of these compounds showed activity, although these were non-toxic even on large dosage.

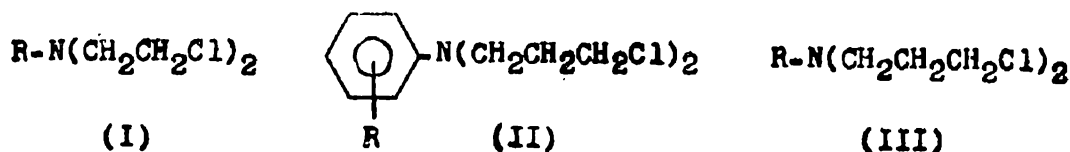
Introduction

In previous papers from our laboratory we have reported the synthesis and anti-tumor activity of Schiff bases from benzaldehyde nitrogen mustards and aromatic amines (1, 2). The biologically and chemically active grouping i.e., alkylating function in these compounds is N, N-bis (2-chloroethyl) amine (I). The representative members of this class which are still used today in clinics for the treatment of cancer are highly toxic to patients. This led the synthetic chemists to make variations in this class of compounds. Ross and co-workers (3) synthesized some of the N, N-bis (3-halopropyl) anilines (II), which are the higher homologues of I.

Although, Popp had synthesized the Schiff bases from benzaldehyde nitrogen mustards and found them active in experimental tumor systems (4), no benzaldehydes

are known carrying the alkylating function of III. We, therefore, found it interesting to synthesize the substituted p-[N,N-bis(3-chloropropyl) amino] benzaldehydes (IV). It is expected that substitutions in the phenyl ring of IV may favour the chemical reactivity of halogen atoms in propyl linkage of alkylating function and thereby enhance the biological activity. Advantage of the formyl group is taken to prepare the Schiff bases VI which might be inactive *per se* but get modified into an active form by processes taking place in the target cells. This approach has been very fruitful in past in search for better antitumor agents (2, 4).

In our earlier work the *m*-haloanilines aminobenzoic acids, 3, 5-dihalo-4-hydroxy-anilines afforded more active Schiff bases with benzaldehyde nitrogen mustards (1, 2). Corresponding Schiff bases from IV have now been synthesized.



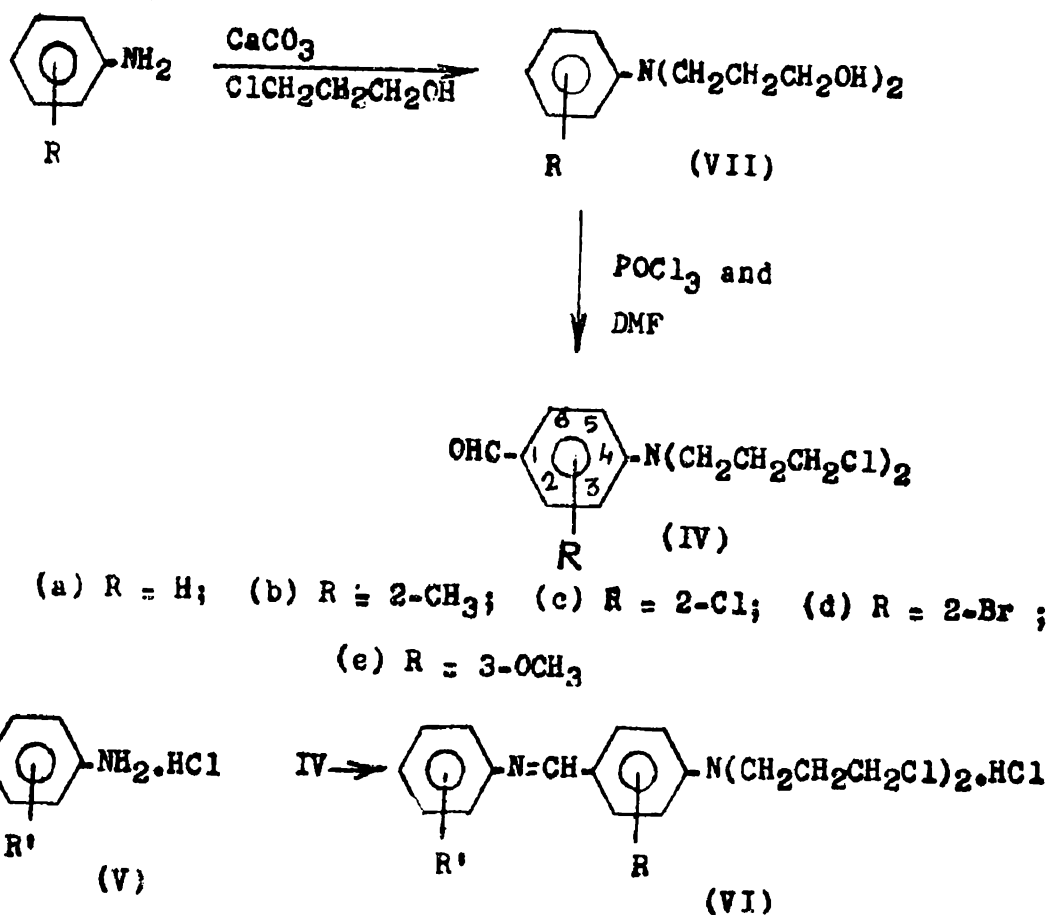
Chemistry

The general method adopted for the preparation of Schiff bases VI (Tables I and II) is the heating of a mixture of amine hydrochloride V and aldehyde in alcohol as shown in Scheme.

N-bis(3-hydroxypropyl) anilines are being reported for the first time.

Experimental

N, N-Bis (3-hydroxypropyl) aniline—Aniline (18.6 g, 0.2 mole) 3-chloropropan-1-ol (56.7 g, 0.6 mole), calcium carbonate



The required *p*-[*N,N*-bis(3-chloropropyl) amino]-benzaldehydes were prepared successfully through the formylation of *N,N*-bis(3-hydroxypropyl) anilines (VII), with POCl_3 in DMF (Dimethylformamide) (4). However, IV (e) was obtained in very poor yield. The *N,N*-bis(3-hydroxypropyl) anilines (VII) were prepared by refluxing the anilines, appropriate quantity of 3-chloropropanal and calcium carbonate in aqueous medium (3). Although, unsubstituted *N,N*-bis(3-hydroxypropyl)-aniline is known in literature (3,5), other substituted *N,*

(20 g, 0.2 mole) and water (250 ml) were placed in a three necked round bottom flask fitted with a mechanical stirrer and a condenser, and the whole mixture was stirred and refluxed. The reaction was continued until all calcium carbonate dissolved. This took about thirty hours. The mixture was then cooled and extracted with CHCl_3 . Chloroform layer was separated and dried over MgSO_4 . Removal of CHCl_3 left a thick oil which upon distillation under reduced pressure gave highly

viscous compound; yield 30 g (71.8 per cent) b.p. 218–225° C/10 mm. (3, 7).

All other substituted N, N-bis (3-hydroxypropyl) anilines were prepared as above and used in subsequent reactions without further purification.

4-[N, N-Bis (3-chloropropyl) amino] benzaldehyde—To dimethylformamide (150 ml) cooled in an ice-bath was added phosphorus oxychloride (33.6 g, 0.225 mole) with stirring at 0–5° C. A solution of N, N-bis (3-hydroxypropyl)-aniline (15.7 g, 0.075 mole) in dimethylformamide (50 ml) was then added to it slowly maintaining the temperature 0–5° C. The mixture was heated in hot water-bath (90–95° C) for two hours and poured on to ice and kept overnight in an ice-box. No solid aldehyde was obtained. The oil that separated, was taken up in ether, the ethereal solution was washed with water, dried over Na₂SO₄ and the ether distilled off to give 77 per cent (16.8 g) of oily aldehyde. When this oily aldehyde was subjected to vacuum distillation, it started to decompose. It was, therefore, characterized and analysed as 2, 4-dinitrophenylhydrazone. 2, 4-Dinitrophenylhydrazone was prepared in usual way and recrystallised from methanol-acetone mixture; had m.p. 198–200° C.

4-[[4-[N, N, Bis (3-chloropropyl) amino]-2-methylbenzylidene]-amino]-2, 6-dichlorophenol hydrochloride—To a solution of 2, 6-dichloro-4-aminophenol hydrochloride

(1.08 g, 0.005 mole) in minimum warm dry ethanol was added a hot ethanolic solution of 4-[N, N-bis (3-chloropropyl)-amino]-2-methylbenzaldehyde (1.44 g, 0.005 mole). The resulting orange coloured solution was boiled for 2-3 minutes. It deposited a crystalline solid on keeping at room temperature. This was collected by filtration, washed with dry alcohol and ether to give 1.70 g (70 per cent) of the hydrochloride salt of the desired Schiff base, m.p. 223–225° C.

Discussion

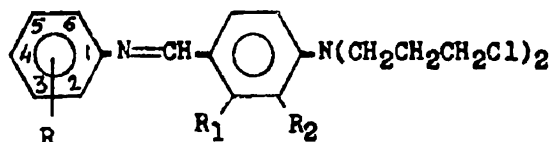
Out of the twenty-two Schiff bases, nine representatives were screened against L-1210 lymphoid leukemia cells implanted intraperitoneally in mice on single dose and chronic schedules. None of these compounds passed the criteria of activity as specified in Cancer Chemotherapy Report (6), however, all of these compounds were found to be non-toxic even at the high dose of 400 mg/kg/day (Table V).

Acknowledgement

The authors are greatly indebted to Dr. H. B. Wood, Chief, Drug Development Branch, National Cancer Institute, Bethesda (U.S.A.) for his co-operation and for making the screening data available and to Mr. M. T. Jaokar for elemental microanalysis. Thanks are due to Dr. B. B. Gaitonde, Director, Haffkine Institute, Bombay, for providing facilities to carry out the present work.

TABLE - I

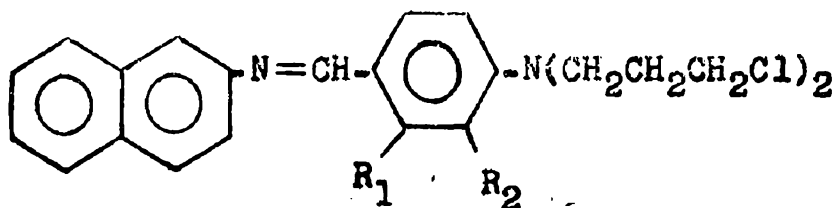
Schiff bases from p-[N,N-Bis(3-chloropropyl)amino]benzaldehydes and aromatic amines.



S.No.	R	R ₁	R ₂	M.p. °C (uncorr) ^a	Molecular formula [*]
1	3-Cl	H	H	182-183	C ₁₉ H ₂₁ Cl ₃ N ₂ .HCl
2	3-Cl	Cl	H	210-212	C ₁₉ H ₂₀ Cl ₄ N ₂ .HCl
3	3-Cl	CH ₃	H	190-192	C ₂₀ H ₂₃ Cl ₃ N ₂ .HCl
4	2,5-Cl ₂	H	H	95-98	C ₁₉ H ₂₀ Cl ₄ N ₂ .HBr
5	3,5-Cl ₂	H	H	197-199	C ₁₉ H ₂₀ Cl ₄ N ₂ .HCl
6	4-Cl	H	H	113-114	C ₁₉ H ₂₁ Cl ₃ N ₂ .HCl
7	4-Cl	CH ₃	H	219-221	C ₂₀ H ₂₃ Cl ₃ N ₂ .HCl
8	3-Br	H	H	105-108	C ₁₉ H ₂₁ BrCl ₂ N ₂ .HCl
9	3-Br	H	OCH ₃	136-140	C ₂₀ H ₂₃ BrCl ₂ N ₂ O.HCl
10	3,5-Cl ₂ ,4-OH	H	H	240-242	C ₁₉ H ₂₀ Cl ₄ N ₂ O.HCl
11	3,5-Cl ₂ ,4-OH	CH ₃	H	223-225	C ₂₀ H ₂₂ Cl ₄ N ₂ O.HCl
12	3,5-Cl ₂ ,4-OH	Cl	H	220-222	C ₁₉ H ₁₉ Cl ₅ N ₂ O.HCl
13	3,5-Cl ₂ ,4-OH	H	OCH ₃	215-218	C ₂₀ H ₂₂ Cl ₄ N ₂ O ₂ .HCl
14	3,5-Br ₂ ,4-OH	CH ₃	H	142-145	C ₂₀ H ₂₂ Br ₂ Cl ₂ N ₂ O.HCl
15	3,5-Br ₂ ,4-OH	H	OCH ₃	223-226	C ₂₀ H ₂₂ Br ₂ Cl ₂ N ₂ O ₂ .HCl
16	4-COOH	Cl	H	212-215	C ₂₀ H ₂₁ Cl ₃ N ₂ O ₂ .HCl
17	4-COOH	H	OCH ₃	165-169	C ₂₁ H ₂₄ Cl ₂ N ₂ O ₃ .HCl
18	2-COOH	Cl	H	132-135	C ₂₀ H ₂₁ Cl ₃ N ₂ O ₂ .HCl

TABLE II

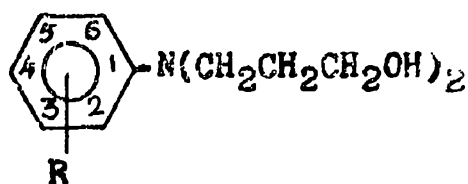
Schiff Bases from p-[N, N-bis (3-chloropropyl) Amino] Benzaldehydes and β -naphthylamine



S.No.	R ₁	R ₂	Melting point °C (uncorrected) ^a	Molecular formula*
19	CH ₃	H	222-225	C ₂₄ H ₂₆ Cl ₂ N ₂ ·HCl
20	Cl	H	215-218	C ₂₃ H ₂₃ Cl ₃ N ₂ ·HCl
21	Br	H	210-212	C ₂₃ H ₂₃ BrCl ₂ N ₂ ·HCl
22	H	OCH ₃	170-172 (Picrate)	C ₂₄ H ₂₆ Cl ₂ N ₂ O·(Picrate)

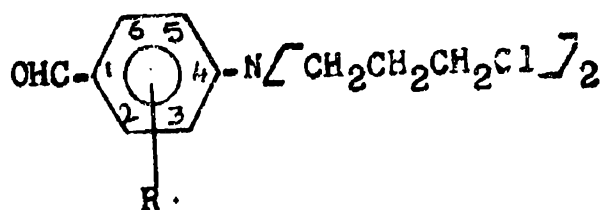
TABLE III

N, N-Bis (3-hydroxypropyl) Anilines



S.No.	R	Yield %	B.p./M.p. °C (uncorrected) ^a	Molecular formula*
1	H	86	218-225/10 mm 59-60 ^b	C ₁₂ H ₁₉ NO ₂
2	3-CH ₃	64	216-222/5 mm	C ₁₃ H ₂₁ NO ₂
3	3-Br	78	235-245/3 mm	C ₁₂ H ₁₈ BrNO ₂
4	3-Cl	80	228-240/3 mm	C ₁₂ H ₁₈ ClNO ₂
5	2-OCH ₃	47	217-225/3 mm	C ₁₃ H ₂₁ NO ₃

TABLE IV

p-[N, N-Bis (3-chloropropyl) Amino] Benzaldehydes

S.No.	R	Yield %	M.p. °C (uncorr)	M.p. °C of 2,4-DNPH** (uncorr.) ^a	Molecular formula [*]
1	H	77	011	198-200	C ₁₉ H ₂₁ Cl ₂ N ₅ O ₄ (2,4-DNPH)
2	2-CH ₃	80	011	172-173	C ₂₀ H ₂₃ Cl ₂ N ₅ O ₄ (2,4-DNPH)
3	3-OCH ₃	11	011	186-188	C ₂₀ H ₂₃ Cl ₂ N ₅ O ₅ (2,4-DNPH)
4	2-Br	64	011	198-200	C ₁₉ H ₂₀ BrCl ₂ N ₅ O ₄ (2,4-DNPH)
5	2-Cl	69	011	204-205	C ₁₉ H ₂₀ Cl ₃ N ₅ O ₄ (2,4-DNPH)
6					

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

(b) Please see Ref. No. 3.

(*) Analysed correctly for C, H and N ± 0.04 %

(**) 2, 4-DNPH : 2, 4-Dinitrophenylhydrazone.

TABLE V

Screening Data against L-1210 Lymphoid Leukemia^{ab}

No. ^c	Dose mg/kg	Survivors	Animal weight difference (gm) (T-C) ^d	Survival days	T/C ^e per cent
1	400	6/6	— 0.4	9.6/9.5	101
2	400	6/6	— 0.4	9.0/9.4	95
	200	6/6	— 0.5	9.6/9.4	102
	100	6/6	— 0.1	9.5/9.4	101
3	400	6/6	— 0.0	9.1/9.4	96
	200	6/6	— 0.1	9.5/9.4	101
	100	6/6	— 0.1	9.5/9.4	101
10	400	6/6	— 1.0	9.6/9.4	102
	200	6/6	— 1.1	9.5/9.4	101
	100	6/6	— 0.6	9.5/9.4	101
11	400	6/6	— 1.1	9.5/9.4	101
	200	6/6	— 2.2	9.3/9.4	98
	100	6/6	— 0.8	9.8/9.4	104
12	400	6/6	— 0.7	9.3/9.4	98
	200	6/6	— 1.0	9.5/9.4	101
	100	6/6	— 0.1	9.8/9.4	104
13	400	6/6	— 0.6	9.0/9.4	95
	200	6/6	— 0.4	9.3/9.4	98
	100	6/6	— 0.6	9.1/9.4	96
14	400	6/6	— 0.1	9.5/9.8	96
	200	6/6	— 0.8	9.4/9.8	95
	100	6/6	— 0.4	9.3/9.8	94
15	400	6/6	— 0.4	10.0/9.8	102
	200	6/6	— 0.1	9.8/9.8	100
	100	6/6	— 0.3	9.6/9.8	97

(a) Only part of the data is presented.

(b) Assays were performed according to specifications established by NCI (formerly CCNSC) as reported in *Cancer Chemotherapy Report*, Part 3, Vol. 3, No. 2 (Sept. 1972).

(c) Numbers refers to those from Tables I and II.

(d) Average weight change of test group (T) minus average weight of control (C) mice.

(e) The index of evaluation for ascitic tumor (for L-1210) is T/C [(mean survival time of treated mice)/(mean survival time of control mice)] × 100. For L-1210 T/C per cent ≥ 125 is considered positive test i.e., compounds demonstrating T/C per cent ≥ 125 are considered active.

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NUTRITIVE VALUE OF 'SUKHADI'— A SUPPLEMENTARY FOOD

H. S. VED, V. P. SHINDE* AND S. D. AMBEGAOKAR

Department of Biochemistry, Haffkine Institute, Parel, Bombay 400 012

A supplementary food 'Sukhadi' distributed by Sadguru Seva Sangh Trust, Bombay, to the drought-affected vulnerable population of various districts of Maharashtra State were analysed for proximate principles, mineral elements and vitamins. It is seen from the results that Sukhadi provides adequate quantity and good quality protein and also can counterbalance the low intake of calories and vitamins. It can be concluded that supplementation of 200 g of Sukhadi/adult/day, as a part of their daily wage equivalent of 50 paise, is certainly helpful to elevate the nutritive value of the daily diet consumed by the adults of drought-affected areas.

Adults from drought-affected areas of various districts of Maharashtra State were provided with constructive work under the 'Drought Relief Programme'. However, among the low income groups of the rural areas, the deficiency of vitamin A, B-vitamins and of iron were commonly occurring signs of malnutrition. The Government of Maharashtra had, therefore, instituted a programme through the Sadguru Seva Sangh Trust, Bombay, to supply 200 g of supplementary food under the name of 'Sukhadi' as a part of wage equivalent of 50 paise. Sukhadi is cooked food prepared from the Protein Enriched Cereal Flour (PECF), jaggery and Vanaspati ghee. The PECF contained 82 per cent High Nutritional Concentrate (HNC). This HNC was prepared from groundnut flour, Bengal gram, horse gram, skim milk powder and lysine along with fat soluble vitamins, B-vitamins, vitamin C and minerals.

The nutritive quality of this supplementary food (Sukhadi) which was distributed in the various districts of the Maharashtra State was assessed by determining the proximate principles, minerals and vitamins. The results of nutritive value of Sukhadi samples are presented and the significance of the same is discussed in this paper.

Materials and Methods

High Nutritional Concentrate (HNC) and its ingredients were collected personally from the cooking centre of Sadguru Seva Sangh Trust, Bombay. At random, two Sukhadi samples from the lot prepared during successive periods and distributed to the drought-affected labourers, were collected by the Collectors of sixteen districts of Maharashtra State and sent to the Haffkine Institute for analysis. The Sukhadi was prepared by cooking the mixture of PECF (55 per cent), jaggery (33 per cent) and Vanaspati ghee (12 per cent). The samples were analysed for their nutritive value. Proximate principles such as moisture, protein, fat, total

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**Present Address*: Testing Unit, Haffkine Institute, Parel, Bombay 400 012.

minerals and carbohydrates were determined by the standard procedures as described in A.O.A.C. (1). Vitamin A was determined spectrophotometrically as per British Pharmacopoeia (2). Vitamin B₁ was determined by converting it to thiochrome derivative according to the method of Association of Vitamin Chemists (3). Aflatoxin was also tested by the method of thin layer chromatography of Haddon *et al.* (4).

Results

Results presented in Table I give the estimated composition of HNC and its ingredients. Aflatoxin was found absent in groundnut flour used in HNC. The results show that more than 50 per cent of protein concentration and minerals content of HNC are contributed by the groundnut flour added in it. Calorific value is also well compensated as in the constituents added. The values of proximate principles and calorific values of Sukhadi samples received from various districts are shown in Table II. The protein concentration and fat content of the Sukhadi samples range from 10 to 15 per cent and 9 to 15 per cent respectively. Sukhadi is also a good source of calories (400 to 433 calories with an average of 416 calories/100 g sample). Minerals (calcium, phosphorus and iron) and vitamins (vitamin A and vitamin B₁) content in Sukhadi are presented in Table III. The ratio of calcium/phosphorus is about 1:2 indicating a proper utilization of these elements for maintaining normal health. The iron content is also fairly good (4 to 8 mg with an average of 6 mg/100 g). Vitamin A (1138 to 1700 I.U. with an average of 1501 I.U./100 g) and vitamin B₁ (273 to 429 µg with an average 354 µg/100 g) in this supplementary food are adequate.

Discussion

It is emphasized (5) that the requirement of protein for animals including man is not merely based on the quantity of the protein but the specific amount of essential amino acids present in it. It is well established by Rose *et al.* (6) that the optimum amount of eight essential amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, tryptophane and valine are required in the diet to maintain the nitrogen balance in adults.

Most of the dietary cereals (jowar, bajra, maize, rice, wheat, nagli, etc.) are low in lysine content and intake of any single cereal is not sufficient to maintain nitrogen balance. If the supplementary food is prepared out of cereal like wheat alone, the deficiency of lysine will be still more pronounced. However, to increase the quality of the dietary protein of the vulnerable groups, HNC is added in the supplementary food. As shown in Table I, HNC is composed of groundnut flour, Bengal gram, horse gram, skim milk powder and vitamins. It is demonstrated (7-9) that these ingredients are very good sources of lysine (5 to 9 g lysine/100 g protein). The supplementation of HNC at 10 per cent level in the Sukhadi increases lysine content and eliminates the need for additional amount of pure lysine; thus the cost of the Sukhadi can be reduced. Moreover, pulses and skim milk powder are good sources of all essential amino acids. Very low methionine content of groundnut flour is well compensated by these. Thus Sukhadi, a supplementary food, can therefore increase the quality of dietary protein.

Protein intake is generally correlated with dietary calories. The protein consumption and calorie intake per day of normal adult in Maharashtra State are 48 g and 1,790 calories against the recommended

allowance 44 g and 2,400 calories respectively (10). Good quality protein consumption and calorie intake of drought-affected persons are generally low due to poor nutritive value of the food consumed; consequently, the famine-affected adults are more susceptible to infection due to protein and calorie deficiencies (11). Considering the above observations, the calorific gap between actual intake and recommended value would be around 800-900 calories. 200 g Sukhadi/adult/day, as a part of their daily wage equivalent of 50 paise, distributed to the vulnerable groups of the drought-affected districts, would provide additional 25 g of good quality protein, which is nearly 50 per cent of the recommended daily allowance. This would also supply extra 850 calories, thus bridging the calorific gap.

Concomitantly, the distribution of Sukhadi to the famine-affected adults can give protection from malnutrition, marasmus and infectious diseases. Cannon (12)

has reported that the antibody production is certainly related to the dietary protein and calorie intake. This supplementary food also provides requisite fat, vitamin A, B-vitamins and minerals to fill up the deficiency gap of the same in the diet.

The nutritional survey and clinical trials, although would have provided interesting observations, the temporary Nutritional Cell was created by the Government of Maharashtra for only two months, in which time, it was difficult to carry out such studies.

Acknowledgement

Authors take pleasure in expressing their thanks to Dr. B. B. Gaitonde, Director, Haffkine Institute, Bombay. Also wish to record their thanks to Government of Maharashtra for creating the temporary Nutrition Cell at the Haffkine Institute and awarding a Senior Research Fellowship to one of us (H.S.V.) to complete the above studies.

TABLE I

Nutritive Value of High Nutritional Concentrate (HNC) and the Ingredients added to it

Food-stuff	Proximate principles (g/100 g)					Calculated calorific value (calories/100 g)
	Moisture	Protein	Fat	Ash	Carbo-hydrates	
Groundnut Flour (deoiled) (<i>Arachis hypogea</i>)	8.6	53.9	0.1	4.2	33.2	349
Bengal Gram (<i>Cicer arictinum</i>)	8.8	24.1	5.1	2.4	59.6	381
Horse Gram (<i>Dolichos biflorus</i>)	8.5	20.1	0.4	3.3	67.7	384
Skim Milk Powder	2.5	37.9	0.1	7.0	52.5	362
High Nutritional Concentrate (HNC)*	8.6	43.3	1.2	4.2	42.7	355

* Composition of High Nutritional Concentrate—Groundnut flour 52 per cent, Bengal gram 19.9 per cent, horse gram 18.0 per cent, Skim milk powder 10.0 per cent and vitamins and lysine 0.1 per cent.

TABLE II

Nutritive Value of Supplementary Food Sukhadi in relation to their Proximate Principles and Calorific Value
(Average values of two different samples)

Serial No.	Districts of Maharashtra State	Proximate principles (g/100 g)					Calculated calorific value (calories per 100 g)
		Moisture	Protein	Fat	Ash	Carbo-hydrates	
1	Ahmednagar	9.4	12.7	13.2	2.8	61.9	417
2	Aurangabad	8.5	15.1	12.7	1.9	61.8	422
3	Bhir	8.9	15.0	11.7	1.7	62.7	416
4	Bombay	8.5	14.4	10.9	1.8	64.4	413
5	Buldhana	9.7	14.7	9.3	1.8	64.5	400
6	Dhule	10.0	13.0	12.3	1.9	62.8	414
7	Kolhapur	7.8	12.4	11.1	2.0	66.7	416
8	Nashik	8.6	14.5	12.5	2.0	62.4	420
9	Usmanabad	9.7	12.4	11.3	2.0	64.6	410
10	Pen	8.7	10.0	13.3	1.8	66.2	424
11	Pune	9.1	13.7	11.7	1.9	63.6	414
12	Ratnagiri	9.9	14.1	12.3	1.9	61.8	414
13	Sangli	9.2	12.6	13.5	2.0	62.7	423
14	Satara	9.4	13.5	12.0	2.2	62.9	414
15	Solapur	8.4	12.1	15.0	2.0	62.5	433
16	Yavatmal	8.2	14.4	10.3	2.0	65.1	411
Average Values ..		9.0	13.4	12.1	2.0	63.5	416

TABLE III

Nutritive Value of Supplementary Food Sukhadi in relation to Mineral Elements and Vitamins
(Average values of two different samples)

Serial No.	Districts of Maharashtra State	Minerals (mg /100 g)				Vitamins (per 100 g)	
		Calcium	Phos-phorus	Iron	Cal. Phos.	Vit A (I.U.)	Vit B (Ug.)
1	Ahmednagar	107	244	5	2.3	1,415	348
2	Aurangabad	96	264	8	2.8	1,693	399
3	Bhir	115	265	8	2.3	1,586	429
4	Bombay	87	225	5	2.6	1,390	299
5	Buldhana	114	250	7	2.2	1,138	344
6	Dhule	121	256	5	2.1	1,482	328
7	Kolhapur	94	220	5	2.3	1,523	347
8	Nashik	87	225	6	2.6	1,406	273
9	Usmaniabad	125	277	8	2.2	1,592	367
10	Pen	108	232	7	2.1	1,700	380
11	Pune	109	227	7	2.1	1,423	370
12	Ratnagiri	102	270	6	2.6	1,375	342
13	Sangli	107	232	5	2.2	1,685	321
14	Satara	82	205	4	2.5	1,628	375
15	Solapur	105	242	6	2.3	1,669	330
16	Yavatmal	111	260	5	2.3	1,321	350
Average Values ..		104.2	243.4	6.0	2.3	1,501	350

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SEROLOGICAL STUDIES IN PLAGUE

D. M. RENAPURKAR, L. V. SARMA, SUSHILA MENON AND M. V. SANT

Zoonosis Department, Haffkine Institute, Parel, Bombay 400 012

Previous serological studies in plague with rat sera from Marathwada showed the possibility of presence of inapparent *Y. pestis* infection in rats. In continuation of this work, haemagglutination tests were undertaken with FI antigen supplied by the WHO to evaluate the possibility of presence of inapparent infection in rats from Marathwada, other districts of Maharashtra State and other domestic animals like cats, dogs and pigs. Two hundred and forty sera of dogs and 198 serum pools from 874 rats from some parts of Maharashtra were examined. Sera of fifteen dogs in Greater Bombay and sera of fifteen pools of rats were positive in titres varying from 1 : 20 to 1 : 160.

Introduction

The passive haemagglutination test developed by Chen and Meyer to detect antibody to Fraction I antigen of *Y. pestis* has proved to be a valuable tool in detecting foci of rodent plague (1). In emphasizing the importance of these serological tests, WHO recommended that serological surveys for plague antibody should be included in every plague surveillance and control programme (2).

Serological tests carried out in some parts of India have shown the possibility of presence of lingering plague foci among rodents (3, 4, 5, 6). These tests were however confined to rodents, and although other animals like dogs have been shown to play a vital part in the epidemiology of plague (7), no attempt was made to screen other animals serologically.

A study was undertaken to evaluate the extent of distribution of antibodies to fraction I antigen of *Y. pestis* in rodents and other animals like cats, dogs and pigs in Greater Bombay and other areas of Maharashtra State. The following account presents the findings.

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Materials and Methods

Rodents were collected from different wards of Greater Bombay, other municipal towns of Maharashtra, and some rural locations. Dog sera were collected from Mahalaxmi dog kennel from stray dogs. Pig sera were collected from Deonar pigsty and cat sera were collected from cats brought to the Pharmacology Department, Haffkine Institute, Bombay.

Antigen

Antigen consisting of tanned erythrocyte sheep cells sensitised to fraction I antigen of *Y. pestis* was supplied by the WHO (Middle-East Asia Research Institute for Plague Control, Moscow, U.S.S.R.).

HA Test

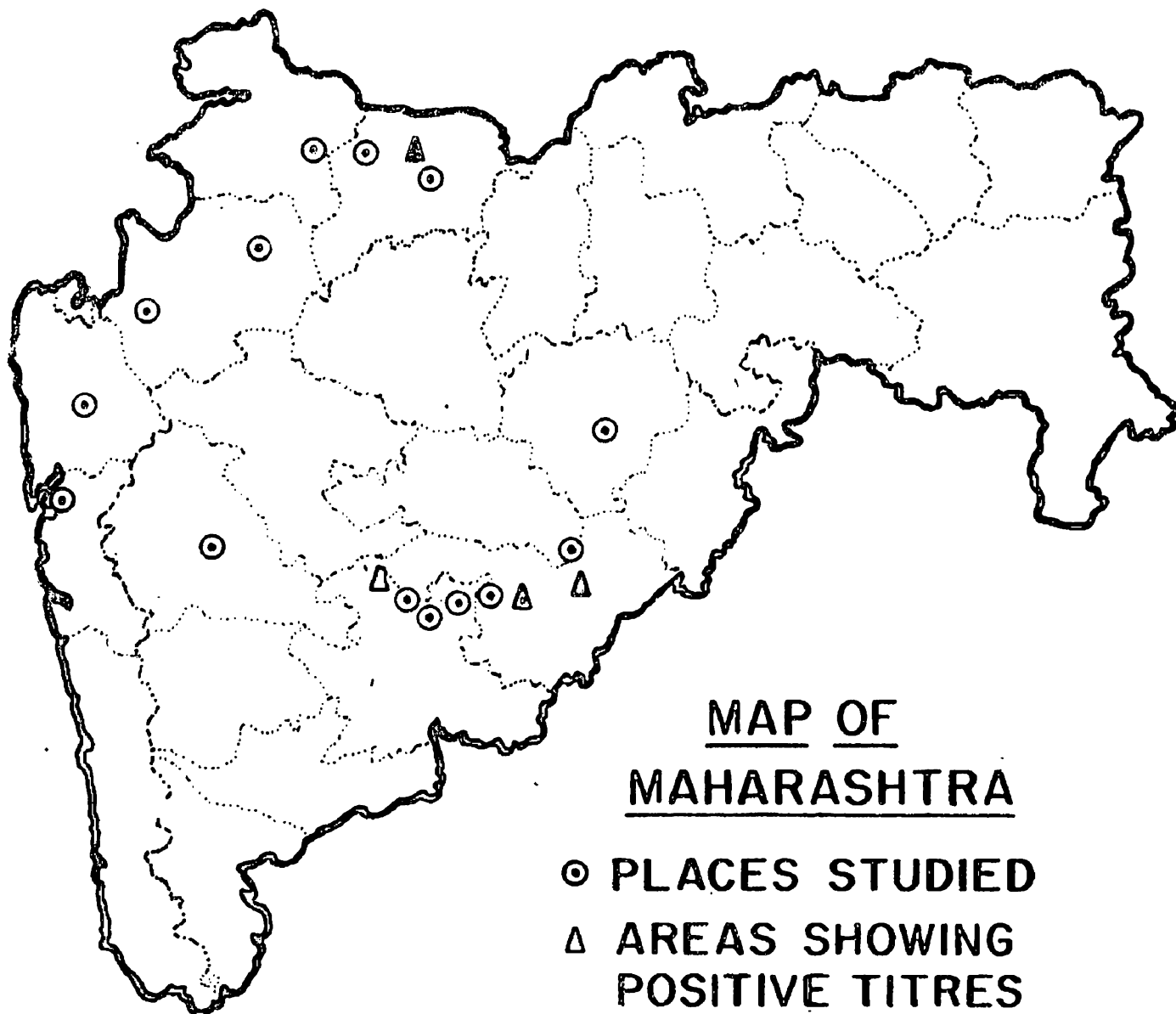
The sera were tested for the presence of haemagglutinating antibodies to the specific fraction I antigen of *Y. pestis* by the methods outlined by Cavanaugh *et al.* (8).

In the case of rodents, pools of serum samples were used. Each serum pool was constituted by sera of five rodents. In Greater Bombay, serological studies were carried out with three species of rodents viz., *R. rattus*, *R. norvegicus* and *B. bengalensis*.

Results

The map of Maharashtra State reproduced here shows the places surveyed for serological tests and the localities showing positive titres.

Murud and Dhoki area (Usmanabad District) and Jalgaon (Jalgaon District). None of the serum pool samples examined from Greater Bombay area showed a positive titre.



In Table I findings of HA tests with rodent sera are presented. In all fifteen serum pools were found to be positive in titres 1 : 20, 1 : 40 and 1 : 160. These serum pools were from Malegaon (Nashik District),

Table II reveals the results of serological tests performed with sera of other animals. It will be seen from the table that only dog sera were found to yield positive titres in good numbers. Three pig sera

were found to be positive in low titres. None of the cat sera examined showed any positive titre.

Discussion

In the present study fifteen out of 198 rodent serum pools exhibited positive titres. These rodent collections were from different parts of Maharashtra State. Apart from Dhoki and Murud areas of Usmanabad District reported earlier (3) two more areas are seen to show positive titres.

Further, although none of the rodent serum pools were positive in Greater Bombay, five per cent dog sera were positive. Rust *et al.* (9) had shown in their

study that 21·6 per cent of dogs showed serological evidence of plague, in contrast to only one out of 1,288 dogs tested in plague free areas. It is not known whether there is any lingering focus of infection that is being transmitted to dogs by contact with infected fleas or by mouthing carcasses of infected rodents.

However, above findings indicate the possibility of existence of lingering foci of plague infection in Maharashtra as pointed out earlier (4, 5, 6) and stress the need for extensive serological and bacteriological survey for undertaking preventive steps to meet any eventuality.

TABLE I

HA Test with Rat Sera

Place	Total number of rodents	Total pools	No. positive/titres				
			1 : 20	1 : 40	1 : 80	1 : 160	Total
Greater Bombay	388	76					
Pune	25	5					
Khed	25	5					
Pachora	32	7	..			..	..
Malegaon	20	4				1	1
Thane	20	4					
Dhule	10	2					
Yedsi	100	20	..	..		..	..
Murud	64	14	4	1		1	6
Nashik	40	10	..	..	..		..
Dhoki	70	15	2	2			4
Barsi	60	12	1				1
Jalgaon	50	10	1				1
Pangri	70	14	2				2
Control +ve 1 : 256							
Total	874	198	10	3		2	15

TABLE II
HA Tests with Sera of Dogs, Pigs and Cats

Place	Species	Number of animals	Titres			Total
			1 : 20	1 : 40	1 : 80	Positive
Greater Bombay	Dogs	240	5	9	1	15
	Pigs	100	3			3
	Cats	7	..	..	..	..
	Total	347	8	9	1	18

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FLUORESCENT STAINING FOR *M. TUBERCULOSIS* IN TISSUE SECTIONS—COMPARISON WITH FITE- FARACO PROCEDURE*

J. K. DAVE, Y. FERNANDES AND S. C. GAWAND

Department of Clinical Pathology, Haffkine Institute, Parel, Bombay 400 012

The results of fluorescent staining method and Fite-Faraco method in identification of *M. tuberculosis* in 243 sections of presumed tuberculous granulomas are presented. The results have indicated that fluorescent staining method is rapid and more sensitive in comparison to the Fite-Faraco method. Hence fluorescence microscopy serves as a valuable adjunct in the diagnosis of problem cases of tuberculous granuloma.

Introduction

The diagnosis of tuberculous granuloma based on the histological criteria alone at times poses a difficult problem to the pathologist. The demonstration of *M. tuberculosis* in tissue section becomes necessary for confirming the diagnosis of tuberculosis in cases of atypical histological findings. The use of fluorochrome dyes in the detection of mycobacteria has been reported far superior to the carbol-fuchsin staining method by several workers (1, 2, 3, 4, 5, 6).

The present paper deals with our experience in the fluorescent staining method for tissue sections of presumed tuberculous granulomatous lesions. The results of fluorescence microscopy were compared with those obtained by modified Fite-Faraco staining method.

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Materials and Methods

The material consisted of formalin-fixed tissues from 243 granulomatous and thirty-five non-granulomatous lesions (Table I). Among the granulomatous lesions, the histological findings were characteristic of tuberculosis in 227 cases, and rather atypical for the diagnosis of tuberculosis in sixteen cases. The non-granulomatous lesions were included to serve as a negative control group.

TABLE I

Selection of the Material

Diagnosis based on histological criteria	Number of cases
Characteristic lesions of tuberculosis	227
Atypical lesions of tuberculosis	16
Non-granulomatous lesions	35
Total	278

The granulomatous tissues were derived from various sites such as lymph nodes, endometrium, intestine, fallopian tube, skin, omentum, etc. (Table II). Additional fresh sections were cut from routinely processed paraffin blocks and stained by (a) a combination of auramine and rhodamine dye for fluorescence microscopy and (b) Fite-Faraco stain with slight modification for detection of acid-fast bacilli by light microscopy.

For fluorescence microscopy, the method of Kuper and May (1960) was followed with slight modifications. A mixture of xylol-peanut oil was used for deparaffinisation. Acid-alcohol was used for decolourisation and potassium permanganate as a counter stain. Tissue of guinea-pigs inoculated with a virulent strain of *M. tuberculosis*, and the smears prepared from *M. tuberculosis* cultures were used as positive controls. Tissue sections devoid of any

TABLE II

Results of Fluorescent and Fite-Faraco Staining Procedures in Characteristic Tuberculous Lesions

Nature of tissue	Auramine total	Auramine +	Per cent	Fite-Faraco total	Fite-Faraco +	Per cent
Lymphnode	110	77	70.0	28	2	7.1
Endometrium	73	53	73.6	36	1	2.7
Fallopian tube	3	2	66.6	3		0.0
Fistular tract	3	1	33.3	2		0.0
Ovary	1		0.0		..	0.0
Omentum	2	2	100.0	2	..	0.0
Intestine	10	7	70.0	3	1	33.3
Spleen	5	4	80.0	3		0.0
Liver	3	2	66.6	3	..	0.0
Kidney	2	1	50.0	2		0.0
Lung	4	2	50.0	4	1	25.0
Subcutaneous tissue	7	6	85.7	5	1	20.0
Myometrium	3	2	66.6	2		0.0
Synovial tissue	1	1	100.0	1		0.0
Total ..	227	160	70.4	94	6	6.3

evidence of granulomatous lesions were used as negative controls. The optical equipment consisted of Leitz microscope with a regular bright-field condenser, Osram HBO 200 high pressure mercury vapour light source together with an appropriate heat absorbing filter, a blue (BG 12) exciter and an orange (OG 1) barrier filter. The sections were scanned with low power objective (10×) using 10× ocular. Typical morphological characteristics of mycobacterium were further confirmed with high power objective (45×).

Freshly prepared sections stained by modified Fite-Faraco stain (AFIP Manual, 1960), (7) were examined for acid-fast bacilli. A minimal survey for fifteen minutes was done with oil-immersion lens (100×) to detect acid-fast organisms in the section before declaring the results negative.

Results

The incidence of positive results in cases of characteristic and atypical tuberculous lesions are shown in Table II and Table III. One hundred and sixty out of a total of 227 cases (70.4 per cent) of characteristic

tuberculous lesion yielded positive results by fluorescent staining method whereas only six out of ninety-four (6.3 per cent) showed acid-fast organisms by Fite-Faraco method.

In the negative control group, the results were negative in thirty-four out of thirty-five non-granulomatous lesions by fluorescence microscopy. The results were entirely negative in the thirty-five sections stained by Fite-Faraco method.

These results clearly indicate the superiority of fluorescent staining method in comparison with Fite-Faraco method in detection of acid-fast organisms in presumed tuberculous granulomas (Table IV).

Discussion

The fluorescent staining procedure is an important tool in the confirmation of tuberculous granulomas particularly in the absence of pathognomonic histological criteria. The superiority of fluorescent staining procedure over the conventional methods has been reported earlier (1, 2, 3, 4, 6). The results of the present study have confirmed the superiority of fluorescent staining procedure.

TABLE III

Results of Fluorescent and Fite-Faraco Staining Procedures in Tissues with Atypical Lesions of Tuberculosis

Site	Auramine staining result			Fite-Faraco staining results		
	Total	Positive	Per cent	Total	Positive	Per cent
Lymphnode	3	1	33.3	3	..	0.0
Endometrium	4	1	25.0	4	1	25.0
Intestine	4	1	25.0	4	..	0.0
Omentum	1	..	0.0	1	..	0.0
Muscle (Psoas)	1	1	100.0	1	..	0.0
Tubo-ovarian mass	1	1	100.0	1	..	0.0
Fallopian tube	1	..	0.0	1	..	0.0
Subcutaneous tissue	1	1	100.0	1	..	0.0
Total ..	16	6	37.5	16	1	6.25

TABLE IV
Comparison between Results of Fluorescent and Fite-Faraco Methods

Diagnosis	Auramine staining results			Fite-Faraco staining results		
	Total	Positive	Per cent	Total	Positive	Per cent
Characteristic lesions of tuberculosis.	227	160	70.4	94	6	6.30
Atypical lesions of tuberculosis	16	6	37.5	16	1	6.25
Total	243	166	68.3	110	7	6.40

Hageman (1937, cited by Mansfield, 1970) (8) first introduced fluorescent dye technique with berberine sulphate for the identification of mycobacteria. Later on he obtained better results using auramine which became a standard fluorochrome stain for mycobacteria (9). Matthaei (10) reported excellent results with the combination of auramine and rhodamine B. Kuper and May (1) further modified the staining procedure using auramine-rhodamine mixture. The method employed herein is that of Kuper and May modified by Mansfield (6).

The staining procedure for fluorescence microscopy is simple. The brilliant orange spots high-lighting the organisms can be detected with low power objective so that the whole section can be rapidly screened. Fite-Faraco method requires screening for a minimum period of fifteen minutes in order to declare the results as negative. The fluorescent staining method would prove useful in the differentiation of tuberculous granulomas from sarcoidosis (5). In cases of atypical histological lesions which are suspicious of tuberculosis, and also in the lesions which appear to be of mixed granulomatous nature, it becomes necessary to detect bacilli in the tissue sections in order to confirm the exact nature of the lesion. In such cases fluorescence microscopy would prove extremely useful.

The specificity of the fluorescent staining method has not been thoroughly investigated. In the absence of the concurrent culture of the biopsy material, it is difficult to opine on the specificity of the staining method. Braunstein and Adriano (2) have shown the superiority of the fluorescent dye technique over the cultural procedure in two cases of tuberculous granuloma.

The rare false-positive results may underscore the diagnostic usefulness of this procedure. Wang (11) has reported a high incidence of false-positive results due to contamination of tissue sections with saprophytic acid-fast bacilli from water baths and paraffin dispensers during the processing of the tissue. Hence it is necessary to take all the precautions avoiding contamination of such nature.

Thus with the availability of the fluorescent microscopy in clinical laboratories today, this method would certainly serve as a valuable adjunct to the histological routine for confirming the diagnosis of doubtful cases of tuberculous granulomas.

Acknowledgement

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BOOK REVIEW

Title : THE BLOOD OF SHEEP : COMPOSITION AND FUNCTION

Editor : BLUNT, M. H., WELLINGTON, NEW ZEALAND

WITH CONTRIBUTIONS BY BLUNT, M.H., COX, R.I., CURTAIN, C.C.,
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E. W., TUCKER, E. M.

*28 figures, 51 tables, XIII, 224 pages, 1975, Cloth D.M. 72, US\$ 31.00
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Review : Sheep has played a significant role as a laboratory animal as well as a domestic animal of great economic value. It is therefore very necessary to know its normal blood values and changes under different physiologic conditions. This monograph fulfils the need of such data. The nine chapters dealing with dynamics of body fluid, cellular elements of the blood, metabolism of carbohydrates and lipids, hormones, various genetic markers and immune system has been written by several authors.

Because of the various ecological conditions and the stress of the climate, altitude and pregnancy, various chapters have dealt with the changes under the different conditions. In number of these studies the data embrace different breeds and the comparative differences for other animal species like goat and also for human beings. Some of the normal values particularly cellular elements of blood, genetic markers, haemoglobins and the immunoglobulins during foetal life, at birth and adult life give the developmental changes of these characters. Similarly the formation of Hb-C under the conditions of anaemia and hypoxia shows the significant importance of Hb-C having better oxygen carrying capacity. Various chapters of the monograph, specially those on dynamics of body fluids, cellular elements of blood, carbohydrate and lipid metabolism and hormones also deal with the changes during pregnancy and lactation. Chapter on the Immune System of the sheep gives normal values of different immunoglobulins, their origin, ontogeny and phylogeny. The last chapter of the monograph deals with the anaemia in the sheep due to parasitic and bacterial infections, deficiency conditions and toxic effects of some of the foods.

The book presents large data concised in a number of tables, charts and figures. The most valuable aspect is the very large bibliography given after every chapter. Dr. Blunt should be congratulated for the compilation of this information monograph.

—Dr. H. M. BHATIA

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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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