

BULLETIN

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TRANSFERABLE ENT AND HLY PLASMIDS IN R PLASMID BEARING ESCHERICHIA COLI FROM NORMAL INDIVIDUALS IN BOMBAY

V. M. RANGNEKAR, D. S. CHITNIS, S. MUKERJI AND S. V. GADRE
Haffkine Institute, Parel, Bombay 400 012.

Extrachromosomal genetic elements designated Ent and Hly control the production of enterotoxin and haemolysin respectively, in *Escherichia coli*. The Ent-positive *E. coli* strains have been implicated as etiological agents of diarrhoea in children, adults, and domestic animals. Although Hly positive *E. coli* have been commonly associated with urinary tract infections in man, the exact role of Hly plasmids in human disease remains unclear. We studied the incidence and genetic stability of Ent and Hly plasmids in R⁺ *E. coli* from normal population in Bombay city. Transferable and genetically stable Ent character was detected in 4.8% R⁺ isolates whereas 11.2% isolates harboured unstable Hly plasmids. None of the R⁺ isolates simultaneously harboured Ent and Hly plasmids.

INTRODUCTION

Enterotoxin production by enteropathogenic strains of *Escherichia coli* is controlled by a transmissible extrachromosomal genetic element designated 'Ent' plasmid (1). Ent positive strains of *E. coli* have been implicated as etiological agents of diarrhoea in children (2,3), adults (4,5) and domestic animals (6,7). A large proportion of the enteropathogenic strains of *E. coli*, particularly of porcine origin, produce alpha-haemolysin, a property borne on another transmissible plasmid designated 'Hly' (8,9). Haemolytic *E. coli* have been commonly associated with urinary tract infections in man and are found in only small proportion of the non-

pathogenic strains inhabiting the gut of man and domestic animals (10,11). However, the exact role of Hly plasmids in human disease is unclear.

It was thought worthwhile to study whether a strain harbouring one or more of these plasmids can persist in an individual without causing clinical diarrhoea thus establishing a carrier state. Our observations are summarized in this communication.

Materials and Methods

Bacterial strains — One hundred and twenty four R plasmid bearing *E. coli* strains were isolated from faeces of normal individuals in Bombay city. These strains were tested for Ent and Hly plasmids.

E. coli K12 (R-Ent-Hly-F-Lac-) carrying chromosomal resistance to 60 mcg/ml nalidixic acid (Nalr) and *E. coli* K12 W 945 (R-Ent-Hly-F-Lac-) carrying chromosomal resistance to 200 mcg/ml streptomycin (Smr) were used as recipients in conjugation experiments to determine the transmissibility of the Ent and Hly character.

Detection of Ent character — The R^+ *E. coli* isolates were examined for enterotoxin production by the adult rabbit ligated ileal loop model (12). Exponential phase cultures of the test isolate were diluted with physiological saline to have a cell concentration of 1.0×10^7 cells/ml, and 1.0 ml of the diluted cultures was inoculated into 10 mm long intestinal loops prepared in adult rabbit. In each animal, positive and negative controls were maintained by inoculating *V. cholerae* 569 B. Inaba culture and plain saline respectively, in separate loops. The rabbit was sacrificed 18 hrs. after inoculation and the fluid accumulated in each loop was compared quantitatively with that in the control loops. Each R^+ *E. coli* culture was tested in three adult rabbits before concluding its enterotoxicity.

Transferability of Ent character — *E. coli* cultures shown to be enterotoxin-positive by above test were screened for conjugal transmission of the Ent character to the recipient *E. coli* strains (listed under *Bacterial strains*) by the method of Smith and Halls

(1). Exponential phase cultures of enterotoxic R^+ *E. coli* (prospective donor) and *E. coli* K12 nalr were mixed in equal proportion in 5.0 ml nutrient broth and incubated at 37°C for 48 hrs., and 0.1 ml of the incubated mixture was inoculated in fresh 5.0 ml nutrient broth and further incubated for 24 hrs. The R^+ transconjugants were selected by plating 0.01 ml and 0.05 ml aliquots of the mixture on nutrient agar containing 60 mcg/ml nalidixic acid and a donor drug marker. At least five colonies of R^+ transconjugants were then tested for Ent character by the ligated intestine test in rabbits. The plasmid nature of the Ent character was established by its conjugal transfer to *E. coli* K-12 Nalr and further to *E. coli* K12 W 945 Smr

Detection of Hly character — The R^+ isolates were tested for haemolysis on sheep blood agar plates (i.e. nutrient agar containing 10% washed sheep-red blood cells).

Transferability of Hly character — The method of examining the plasmid nature of the haemolysin-producing property was the same as that described for detecting Ent plasmids, except that aliquots of the mating mixture were plated on sheep blood agar plates containing nalidixic acid and donor drug markers. After incubation at 37°C for 24 hrs., the plates were examined for R^+ transconjugant cell colonies showing partial haemolysis.

Genetic stability of Ent and Hly plasmids — The R^+ *E. coli* isolates bearing Ent and Hly plasmids were subcultured daily in nutrient broth upto fifteen subcultures and then re-examined for Ent and Hly character and its transferability.

RESULTS

Incidence and transferability of Ent and Hly character — Of the 124 R^+ *E. coli* isolates examined, ten (8.06%) caused fluid accumulation in the intestinal loops. Six of these enterotoxic strains transmitted the Ent character by conjugation to *E. coli* K12 recipients. Fourteen (11.2%) of the 124 R^+ isolates harboured the Hly character. All these strains were able to transmit this character to recipient *E. coli* K12 strains indicating its plasmid nature.

It was noted, however, that none of the original 124 R^+ isolates carried both Ent and Hly plasmids, simultaneously.

Stability of Ent and Hly character — The enterotoxigenic character was genetically stable in the *E. coli* isolates even after fifteen subcultures in nutrient broth, despite the fact that R plasmids from four of these isolates were lost during subculture. The other six Ent + R^+ isolates could transfer their plasmids to the recipient strains by conjugation. On the other hand, the Hly plasmids were highly unstable and all the Hly+ isolates were rendered Hly- within fifteen subcul-

tures; only five of these also lost their R plasmids during this period.

DISCUSSION

In this investigation, the incidence of Ent and Hly plasmids was observed to be low. It should be noted, however, that this study was limited to R plasmid bearing *E. coli* from normal individuals and the actual incidence of the enterotoxic and haemolytic strains of *E. coli* in the gut of individuals might be higher.

The transfer factor of R plasmids can also possibly mobilize the Ent and Hly plasmids during conjugation (13,14,15). However, in case of four of our enterotoxic *E. coli* isolates the enterotoxigenicity was not transferable even in the presence of R plasmids. This could be either due to the chromosomal location of the genes coding enterotoxin production or due to the inability of the existing R factors to promote the transfer of Ent markers.

None of the R^+ isolates harboured both Ent and Hly plasmids, simultaneously. Moreover, our stability studies revealed that the Ent plasmids were genetically stable, whereas the Hly plasmids were lost within fifteen subcultures. Genetically stable as well as unstable Ent and Hly plasmids have been reported earlier (1,9,16,17). These observations could probably be explained if compatibility between the Ent, Hly and R plasmids is studied extensively.

Although the role of Hly plasmids in human disease is unclear, the Ent plasmids are known to be responsible for conferring enteropathogenicity upon *E. coli* hosts. In spite of this, none of the individuals from whom these strains were isolated suffered from diarrhoea. Perhaps, the proportion of such strains in the gut was never sufficiently high to initiate diarrhoeal disease or the ecology of the gut restricted their enteropathogenicity.

The significance of the associa-

tion of Ent and Hly plasmids with R plasmids in *E. coli*, particularly with respect to their role in human disease, is poorly conceived. Whether these plasmids afford any selective advantage to their host and whether antibiotic treatment is advisable against diarrhoea, remains a matter of discussion.

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SYNTHESIS AND STUDY OF 2,4 DIAMINO 6-SUBSTITUTED-ARYLIDENO AND BENZAMIDO QUINAZOLINES AS POSSIBLE ANTITUBERCULOSIS AGENTS : I

B. G. KHADSE, S. R. LOKHANDE, MADHUBEN R. PATEL AND
V. L. KHAIRNAR,
*Department of Chemotherapy, Haffkine Institute, Parel,
Bombay 400 012.*

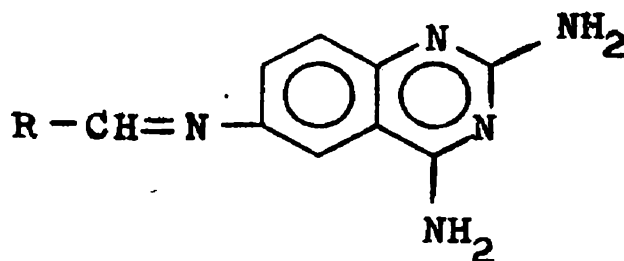
Two different series of 2, 4-diaminoquinazolines bearing an aryl function attached to the 6-position through an azo-methine linkage and another through amido-linkage were synthesised and studied for their *in vitro* antituberculosis activity against *M. tuberculosis* (H₃₇Rv strain).

INTRODUCTION

Antibacterial activity was shown by a number of amino quinazolines (1). In recent communications also, it was reported that 2,4-diamino-6-[(arylkyl and heterocyclic)-methyl]-amino quinazolines, exemplified by 2,4-diamino-6-[(3,4-dichlorobenzyl) amino]-quinazoline (2) exhibit strong antimalarial as well as antibacterial activity. Similarly, compounds containing amino groups at both 2

and 4 positions of quinazoline afforded optimum inhibitory activity and an aromatic substituent at 6-position caused substantial increase in inhibitory potency (3).

Different types of Schiff bases reported (4) from our laboratory possesses good antituberculosis activity. These observations lead us to prepare Schiff bases from 2,4,6-triamino quinazoline of the following type (I).

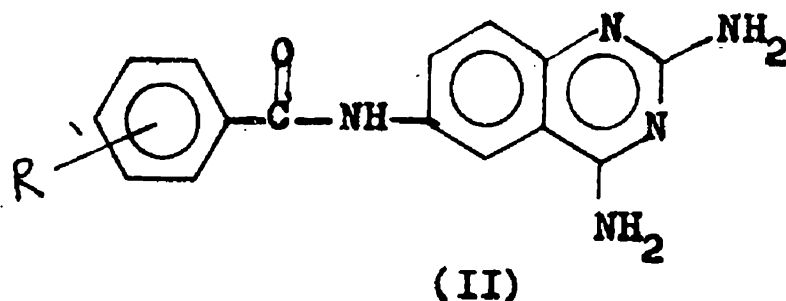


(I)

Where, R - Substituted-aryl & heterocyclic.

In addition, few 2,4-diamino-6-(substituted - benzamido)-quinazolines (II) were synthesised and

studied for their antituberculosis activity.



Where, R = H, Cl, Br, I, OC₂H₅ ;
2,4-Cl₂ and 3,4-Cl₂.

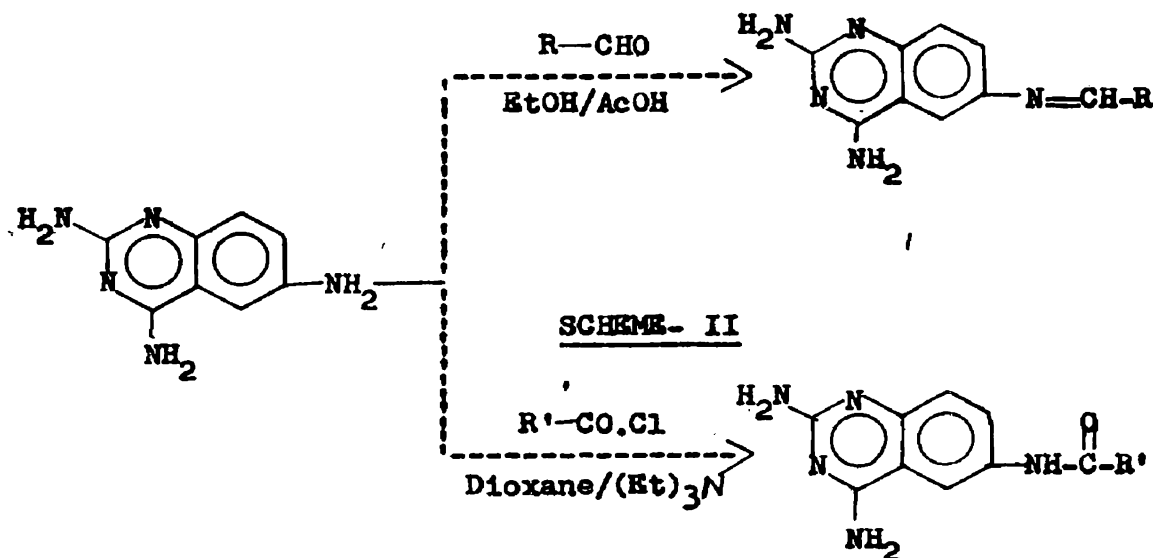
CHEMISTRY :

The synthetic procedure utilized for the preparation of Schiff bases (I) is outlined in *Scheme I*. Thus, the 2,4,6-triaminoquinazoline was condensed with the appropriate benzaldehydes or heterocyclic aldehydes in ethanol in the presence of a drop of acetic acid as catalyst to give the Schiff base.

2,4-diamino-6-(substituted - benzamido)-quinazolines (II), were prepared by the condensation of 2,4,6-triamino-quinazoline and sub-

stituted benzoyl chlorides in non-polar solvent in the presence of triethylamine, are outlined in *Scheme II*. The requisite 2,4,6-triamino quinazoline was obtained by the reduction of the corresponding 2,4-diamino-6-nitroquinazoline with stannous chloride according to the method of Davoll *et al.* (5). Condensation of the aldehydes or acid chlorides with the 6-amino group of the 2,4,6-triamino-quinazolines preferentially take place due to its aromatic character.

SCHEME -I



Where,

$R \approx$ Substituted-aryl, heteroaryl

$R' \approx$ Substituted aryl.

EXPERIMENTAL :

General synthetic procedures for

(a) *Schiff bases*: A mixture of 0.001 mole of 2,4,6-triamino quinazoline and 0.001 mole of appropriate aldehyde, was taken in 20 ml of ethanol with a drop of acetic acid. This mixture was refluxed on steam bath for 3 hours. After cooling the reaction mixture, crystalline solid of required Schiff base was obtained and recrystallised from DMF.

(b) *2,4-Diamino-6-(substituted-benzamido) quinazolines*:

A 0.001 mole of substituted benzoyl chloride was taken in 10 ml

of dioxane and added dropwise to a solution of 0.001 mole of 2,4,6-triamino quinazoline in 20 ml of dioxane containing 0.001 mole of triethylamine, below 25° . It was stirred for 4 hours. The precipitated amide derivative was washed with $NaHCO_3$ solution. Recrystallised from ethanol.

Similarly, all other compounds reported in Tables I and II were prepared by the appropriate method.

BIOLOGICAL RESULTS

Serial dilution method of Doub and Youman (6) was used to screen these compounds for in

vitro antituberculosis activity against H₃₇Rv strain of *M. tuberculosis*, cultivated in Youman's medium containing 10% horse serum.

Mostly all Schiff bases (Table I) were found to be inactive, at 200 µg/ml conc. except compound 1 which inhibit the growth of *M. tuberculosis* at 6.25 µg/ml conc. and compound 6 which was active at 50 µg/ml. Among, the another series of 2,4-diamino-6-(substituted benzamido)-quinazolines (Table

II) only compound No. 7 was active at 25 µg/ml.

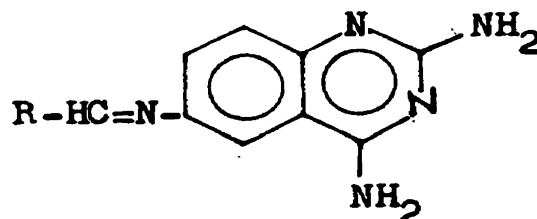
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TABLE I

Schiff bases

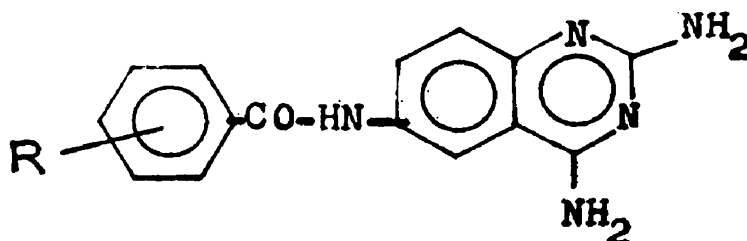
No.	R	Yield %	M.p. ^a (O _c)	Mol. formula ^b	M I C ^c (μg/ml)
1.	2,6-Dichloro-4-hydroxyphenyl	80.	> 320	C ₁₅ H ₁₁ Cl ₂ N ₅ O	6.25
2.	4-Acetamidophenyl	64	247	C ₁₇ H ₁₆ N ₆ O	Inactive
3.	3-Methoxy-4-hydroxy-phenyl	75	196	C ₁₆ H ₁₅ N ₅ O ₂	„
4.	2-Hydroxy-5-bromo-phenyl	79	316	C ₁₅ H ₁₂ BrN ₅ O	100
5.	2-Hydroxy-5-chloro-phenyl	67	> 320	C ₁₅ H ₁₂ ClN ₅ O	Inactive
6.	3-Bromo-4-Hydroxy-5-methoxyphenyl	73	210	C ₁₆ H ₁₄ BrN ₅ O ₂	50
7.	2-Thienyl	69	261	C ₁₃ H ₁₁ N ₅ S	Inactive
8.	3-Pyridyl	70.5	237	C ₁₄ H ₁₂ N ₆	„
9.	5-Nitro-2-furyl	58	> 340	C ₁₃ H ₁₀ N ₆ O ₃	„
10.	N ¹ -Methyl-N ⁴ -piper-azinyl	65	231	C ₁₄ H ₁₉ N ₇	„

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

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

TABLE II

2, 4-Diamino-6-(Substituted benzamido)-quinazolines



No.	R	Yield %	M.p. °C ^a	Mol. formula ^b	M I C ^c (μg/ml)
1.	H	65	306	C ₁₅ H ₁₉ N ₅ O	Inactive
2.	4-Chloro	51	265	C ₁₅ H ₁₂ ClN ₅ O.H ₂ O	„
3.	4-Bromo	45	> 340	C ₁₅ H ₁₂ BrN ₅ O	100
4.	4-Floro	48	285	C ₁₅ H ₁₂ FN ₅ O	Inactive
5.	4-Iodo	37	> 340	C ₁₅ H ₁₂ IN ₅ O.H ₂ O	100
6.	2, 4-Dichloro	50	250	C ₁₅ H ₁₁ Cl ₂ N ₅ O.H ₂ O	Inactive
7.	3, 4-Dichloro	52	210	C ₁₅ H ₁₁ Cl ₂ N ₅ O	25
8.	4-Ethoxy	51	250	C ₁₇ H ₁₇ N ₅ O	Inactive
9.	2-Hydroxy-5-nitro	42	212	C ₁₅ H ₁₂ N ₆ O ₄	100

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

(b) All compounds were analysed for C, H, N and found within $\pm 0.4\%$ of theoretical values.(c) Minimum inhibitory concentration for *in vitro* antituberculosis activity (for I N H = 0.04 μg/ml; and for PAS = 0.4 μg/ml).

PRELIMINARY STUDIES ON ANTIFERTILITY ACTIVITY OF *LYGODIUM FLEXOSUM* (LINN.) IN ALBINORATS-1

R. T. MAHAJAN, B. B. GAITONDE, V. B. BABNE AND I. V. BADE

Director's Research Cell, Haffkine Institute, Parel, Bombay-400 012.

Investigations were carried out on the plant *Lygodium flexosum* (Linn) as it has a reputation in folklore to possess antifertility activity. The crude aqueous extract of the plant rhizomes had maximum antifertility activity, the leaves and stems also possessed substantial activity. Alcohol extract and aqueous extracts of rhizomes were prepared and fed to rats orally. All animals fed on the alcohol extract remained sterile, while 70% of those fed on water extract showed infertility. The petroleum ether extract had almost negligible antifertility activity.

INTRODUCTION

A number of indigenous plants have been reported to possess the property of preventing birth when administered orally. De Laszlo and Henshaw (1) have mentioned 60 plants used in folklore medicine as contraceptives of which 39 were of Indian origin. Indian folklore and Ayurvedic systems of medicine mention the use of some plant products as local as well as oral contraceptives. In the present investigation we selected *Lygodium flexosum* because of its reputation as an antifertility agent in folklore medicine. This plant is being used by Adivasis in the Western part of Maharashtra State and in Gujarat for "inflicting punishment" on the enemy's women folk by rendering them permanently sterile. *Lygodium flexosum* is a small genus of Schiaezoid fern characterized by

climbing leaves. It belongs to family Schizaeaceae, and is ephemeral, available only during monsoon.

MATERIALS AND METHODS

The plant material was collected from Thana District in Maharashtra State. It was dried at room temperature and then powdered using a pulverizer. One kg. of dried material was successively extracted with four litres each of petroleum ether (60-80°C) and 95% alcohol by soxhlet apparatus. The aqueous extract was prepared by treating the material with excess of water at 50-60°C in water bath over a period of 6-8 hours. The petroleum and alcoholic extracts so obtained were filtered to remove any suspended matter and solvents were removed under reduced pressure at 50-60°C on a flash evaporator. The aqueous extract was

concentrated on water bath by slow evaporation at 50-60°C. The requisite quantity was administered orally as a suspension made with gum acacia. Control animals received only vehicle in appropriate doses.

Proved fertile albino rats, of either sex, weighing between 180-250 gms. (5-7 months), used in this study were obtained from Haffkine Bio-Pharmaceutical Corporation Limited, Bombay. All animals were given the standard diet and water *ad lib*.

Proved fertile female rats (fertility in our animal house is almost 90-95%) were successfully mated with proved fertile males in proestrus phase of the estrus cycle. The ratio of animals kept in a single cage was three females : one male. The day on which thick clumps of spermatozoa were observed in the vaginal smear was considered as day 1 of pregnancy. The plant extracts were administered orally by means of a syringe and a feeding needle from day 1 to day 7 of pregnancy. On day 11 of pregnancy, the animals were laparotomized by an abdominal incision under light ether anaesthesia. The uterine horns were examined and the number of implantation sites in both the horns were recorded. After examination the abdomen was closed in the usual way and the animals were returned to their respective cages. The antifertility activity was assessed by the method described by Khanna and Choudhary (2).

RESULTS

Table 1 shows the antifertility effect of the petroleum ether, alcoholic and aqueous extracts of *Lygodium flexosum* administered orally in different doses to successfully mated female albino rats.

The petroleum ether extract in doses upto 500 mg/kg orally was found to be ineffective. The aqueous extract, however was partially (70%) active in the same dose. The alcoholic extract was the most potent of the three and completely inhibited implantation in all animals treated with an oral dose of 500 mg/kg body weight.

DISCUSSION

Plant materials are a rich source of biodynamic agents of all types. Practically every pharmacological category of synthetic drugs has its prototype in nature i.e. a substance of natural origin. According to the Ayurvedic principle, every plant on the surface of the earth can be used as a medicine. Thus, it is not unreasonable to believe that the plant kingdom should have an effective antifertility drug in its huge armamentarium (3). Many tribes in various parts of our country (4,5) as well as in different parts of the world have been known to use certain plants for the prevention of conception (3,6). The selection of the indigenous plant for antifertility screening in the present study was mainly based upon its use as an antifertility agent in folkore medicine.

TABLE I

Antifertility activity of different extracts of Rhizomes
of *Lygodium flexosum* (Linn) in female rats

Treatment	Dose mg/kg	No. Pregnant No treated	Average no. of implantation sites per rat Mean $\pm$ S. E.	Average litter size Mean $\pm$ S.E.	Antifertility effect %
Control	Gum acacia 5%	10/10	9.30 $\pm$ 0.45	8.00 $\pm$ 0.36	Nil
Petroleum	100	10/10	8.80 $\pm$ 0.53	7.80 $\pm$ 0.42	Nil
ether	250	10/10	8.20 $\pm$ 0.62	7.40 $\pm$ 1.02	Nil
extract	500	8/10	7.37 $\pm$ 0.46	6.50 $\pm$ 0.36	20
Alcoholic	100	9/10	7.70 $\pm$ 0.97	6.60 $\pm$ 0.82	10
extract	250	5/10	5.14 $\pm$ 0.50	3.70 $\pm$ 0.48	50
	500	0/10	0	0	100
Control	Dist. Water	10/10	9.00 $\pm$ 2.47	7.70 $\pm$ 1.53	Nil
Aqueous	100	10/10	8.60 $\pm$ 0.71	7.40 $\pm$ 0.47	Nil
extract	250	8/10	7.00 $\pm$ 0.81	6.00 $\pm$ 0.69	20
	500	3/10	4.00	3.30	70

This drug exerts its antifertility effect during the early stages of pregnancy. It is evident from our results that this plant contains an active ingredient (S) which exhibits antifertility effects. The inability of petroleum ether extract to demonstrate the antifertility activity shows that the active ingredient is polar, it can be extracted in polar solvents like

alcohol and water. To the best of our knowledge no work on phytochemical study has been done so far on this plant genus. The results of our detailed work on separation and fractionation of alcoholic extract to find out the chemical nature and probable mechanism of action of this drug will be reported in our next communication.

ACKNOWLEDGEMENT

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VARIABILITY IN THE TOXICITY OF THE VENOMS OF INDIAN LAND SNAKES

S. BHATTACHARYA, S. S. JADHAV AND B. B. GAITONDE

Director's Research Cell, Haffkine Institute, Parel, Bombay -400 012.

The toxicity variation in the various samples of the venoms of four snakes (i) cobra (*Naja naja*) (ii) krait (*Bungarus caeruleus*) (iii) russell's viper (*Vipera russelli*) and (iv) saw scaled viper (*Echis carinatus*) used for antivenin production has been studied. It was found that except in the venom of the krait, there was a marked variation in the toxicities of the samples of the other three venoms. Our data suggests that this variation is due to conditions during drying and processing and not because of storage. It is suggested that greater care should be taken during processing and drying of venoms.

INTRODUCTION

Snake venoms are complex mixtures of toxic and non-toxic proteinaceous and non-proteinaceous substances. The toxicity of any snake venom sample is dependent on many factors like age, sex, nutritional status of the snake, seasonal variations, time gap between two successive milkings, geographical distribution, etc. (1,2). This variation in the toxicity poses problems in the standardization of venom samples. The convenient way to overcome this would be by pooling together a large number of samples of venoms obtained during various seasons and then using the pooled venom sample for standardization. Factors such as housing conditions of the snakes, their milking and the processing and storage conditions of the

venom are major determinants of the potency of a venom sample.

Snake venoms are rich in many hydrolytic enzymes. Among these are proteinases, abundantly found in viperid and crotalid venoms, which significantly modify the shelf life of a venom sample. This is because they act on toxic polypeptides and break them down to smaller non-toxic peptides. The action of these proteinases are therefore of great significance in the establishment of a venom standard. Viperid and crotalid venoms, on the other hand, tend to be highly unstable on storage, especially in aqueous solution. Elapid venoms, which are devoid of proteinases are thus expected to be more stable.

Until about a decade ago, venoms were dried in desiccators,

using calcium chloride, at around 4°C and then stored as dry powder at a low temperature. This process, being lengthy, was detrimental to the stability of the venoms and comparison of the toxicity data of various laboratories and often of the same laboratory at different periods was indeed difficult. Taborska (3) highlights some of these problems faced by toxinologists. With the introduction of the process of lyophilization, the variability induced during the drying process has been substantially minimized.

Many laboratories like Haffkine Institute, and Serpentaria, pool together the venom samples collected during a complete milking session of one week of one species of snake, distribute it into vials and store in refrigerators at 4°C before lyophilization. The time which elapses between the milking of a snake and the freezing of its venom tends to be quite long.

Another factor which may affect the toxicity, because of storage of venoms in the liquid form is the cellular debris that settles on standing. When such a sample is distributed into vials, the portions which are drawn from the bottom tend to contain a greater proportion of non-toxic cellular debris. This would result in toxicity variations in samples of venom drawn from the same batch of venom.

The present investigation was planned in order to study the variations in the toxicity of snake venoms and also the effect caused

by physical factors during drying and storage. The project was undertaken with a view to standardize venom and subsequently antivenin.

MATERIALS AND METHODS

Venoms of the four species of snakes (i) cobra (*Naja naja*) (ii) Krait (*Bungarus caeruleus*); (iii) russell's viper (*Vipera russelli*); and (iv) saw scale viper (*Echis carinatus*) were obtained from the snake farm of the Haffkine Institute, Bombay in the form of lyophilized, dry powder. These powders were obtained from venom samples collected in various times of the year and in various years. In the case of *Echis carinatus* venom, venom has to be collected during only one season (between August and December) as these snakes are collected in this season only and do not live very long in captivity. One sample of *Echis carinatus* venom was obtained fresh after milking, immediately frozen at -40°C and lyophilized.

Toxicity of the venoms was determined by injecting various doses, in volumes of 0.5 ml, intravenously into mice (Kasauli strain, 18-20 gms) obtained from Haffkine Bio-pharmaceutical Corporation Limited. The LD₅₀, and its confidence limits, were calculated for each batch of venom as per the method of Litchfield and Wilcoxon (4).

RESULTS

Table I gives the values of LD₅₀ of different batches of cobra

TABLE — 1

Data on the LD₅₀ of different batches of cobra venom in mice

Lot. No.	Date of milking	Date of lyophilization	Time lapse (days)	LD ₅₀ (ug/mouse)	Range*
1	17.11.75	12.12.75	25	8.0	6.95 - 9.20
2	20.04.75	16.06.76	57	8.1	6.92 - 9.47
3	31.08.76	04.11.76	65	15.2	12.56 - 18.39
4	31.08.76	09.11.76	70	15.3	13.42 - 17.44
5	22.02.77	10.03.77	15	4.5	4.05 - 4.99

Mean = 10.22 ± 2.15.

*Range is between 95% confidence limits.

TABLE — 2

Data on the LD₅₀ of different batches of krait venom in mice

Lot. No.	Date of milking	Date of lyophilization	Time lapse (days)	LD ₅₀ (ug/mouse)	Range*
1	25.06.76	13.07.76	18	1.9	0.95 - 2.13
2	22.02.77	18.03.77	24	2.0	1.75 - 2.28
3	24.12.76	28.12.76	4	2.1	1.86 - 2.37
4	21.10.74	6.11.74	16	2.4	2.07 - 2.81

Mean = 2.1 ± 0.08.

*Range is between 95% confidence limits.

TABLE — 3

Data on the LD₅₀ of the various batches of Echis carinatus venom in mice

Lot. No.	Date of milking	Date of lyophilization	Time lapse (days)	LD ₅₀ (ug/mouse)	Range*
1	10.06.65	N.R.	—	12.4	10.87 - 14.14
2	15.09.75	30.10.75	45	13.7	11.42 - 16.44
3	12.06.75	N.R.	—	12.6	10.95 - 14.49
4	25.08.76	15.09.76	21	14.2	12.04 - 16.75
5	29.11.76	9.12.76	10	11.8	10.00 - 13.92
6	17.08.77	24.08.77	7	13.1	10.92 - 15.72
7	28.09.77	12.10.77	14	12.8	9.84 - 16.74
8	16.10.77	16.10.77	0	9.0	7.62 - 10.62

Mean = 12.45 ± 0.56.

*Range is between 95% confidence limits.

N.R. Not Recorded.

TABLE — 4

Data on the LD₅₀ of the various batches of Russel's viper venom in mice

Lot. No.	Date of milking	Date of lyophilization	Time lapse (days)	LD ₅₀ (ug/mouse)	Range*
1	25.03.55	4.04.55	9	5.1	4.63 - 5.61
2	14.12.76	17.12.76	3	5.3	5.22 - 6.44
3	4.04.78	25.04.78	21	5.6	5.10 - 6.18
4	25.05.78	7.06.78	13	8.6	7.83 - 9.47

Mean = 6.15 ± 0.83.

*Range is between 95% confidence limits.

venom. There was a large variation seen in the LD_{50} values. In lot no. 5, where the time lapse between milking and lyophilization was 15 days, the LD_{50} was the lowest ($4.5 \mu\text{g}/\text{mouse}$ or $0.225 \text{ mg}/\text{kg}$) while in case of lot no. 3 and 4 where the time lapse was greatest (65 and 70 days), the LD_{50} value was the highest (15.2 and $15.3 \mu\text{g}/\text{mouse}$ or 0.760 and $0.765 \text{ mg}/\text{kg}$). The mean LD_{50} value of the five lots chosen was $10.22 \pm 2.15 \mu\text{g}/\text{mouse}$ or $0.511 \pm 0.107 \text{ mg}/\text{kg}$ (Mean $\pm$ S.E.). The value of the standard error makes it evident that there is a significant variation in the toxicity of various lots of crude venoms. Also it can be seen that the ranges of 95% confidence limits for the lots fall into three discrete groups, where lot no. 5 stands out significantly ($P < 0.05$) more toxic than the other batches, lot nos. 1 and 2 are of similar toxicity and are significantly ($P < 0.05$) more toxic than lot nos. 3 and 4, which are similar, and the least toxic. However, it is apparent that the variation is predominantly due to factors during drying and not during storage, since older batches were not necessarily less toxic than the newer ones.

Table 2 summarises our data on krait venom. There is no significant ($P > 0.05$) difference in the toxicities of the various batches of venom. The most toxic lot was lot no. 1 ($LD_{50} = 1.9 \mu\text{g}/\text{mouse}$ or $0.095 \text{ mg}/\text{kg}$) and the least toxic was lot no. 4 ($LD_{50} = 2.4 \mu\text{g}/$

mouse or $0.120 \text{ mg}/\text{kg}$). The mean LD_{50} of these four batches was $2.1 \pm 0.08 \mu\text{g}/\text{mouse}$ or $0.105 \pm 0.004 \text{ mg}/\text{kg}$ (Mean $\pm$ S.E.).

Data on the venom of *Echis carinatus* is presented in table 3. The sample of venom which had been immediately frozen and lyophilized had a higher toxicity than the others. A time lapse of 10-40 days at 4°C before lyophilization caused a marked change in the toxicity. The most toxic of the batches was lot no. 8 ($LD_{50} = 9.0 \mu\text{g}/\text{mouse}$ or $0.450 \text{ mg}/\text{kg}$) and the least toxic was lot no. 4 ($LD_{50} = 14.2 \mu\text{g}/\text{mouse}$ or $0.710 \text{ mg}/\text{kg}$). There was no significant ($P > 0.05$) difference between the LD_{50} of the sample collected in 1965 and those collected in 1975, 1976 or 1977.

In the case of the Russell's viper venoms marked differences were observed between the various batches of venom tested. The most toxic lot lot no. 1 was ($LD_{50} = 5.1 \mu\text{g}/\text{mouse}$ or $0.255 \text{ mg}/\text{kg}$) and the least toxic was lot no. 4. ($LD_{50} = 8.6 \mu\text{g}/\text{mouse}$ or $0.430 \text{ mg}/\text{kg}$). The mean LD_{50} values was $6.15 \pm 0.83 \mu\text{g}/\text{mouse}$ or $0.304 \pm 0.0415 \text{ mg}/\text{kg}$. (Mean $\pm$ S.E.).

DISCUSSION

Changes in the electrophoretic and immunological properties of snake venom due to drying and storage conditions have been reported by many workers for many years (5,6,7). It is generally accepted that changes in biological properties occur mainly during drying and not during storage (8,9,10,11).

As against these reports, however, considerable loss of lethal toxicity has been reported during the storage of desiccated and freeze dried snake venoms (11,12). Coagulant and thrombin-like activities were reported to be present in fresh Gaboon viper venom but not in four commercial preparations of the same venom (13). Willemse and Hattingh (14) studied the biological properties of *Hemachatus hemachatus* venom and found that although freshly freeze dried venom was more potent than desiccated commercial preparations, it deteriorated to the latter condition on storage. The results of the present investigation are therefore to be seen in the light of such controversial data. Our results on the toxicity variation seen in cobra venom and the two viper venoms show that the time which elapses between milking and lyophilization is of crucial importance in the determination of the toxicity of a sample of venom. Our results support the work of others (8,9,10,11) but are apparently contradictory to the data obtained by Willemse and Hattingh (14). These authors had not mentioned in the above paper the exact conditions under which their venom samples were stored so that it becomes impossible to comment on their results vis-a-vis those of the present investigation.

Krait venom is relatively stable, probably because of the absence of proteinases in this venom. We have observed (Gaitonde *et al*, unpub-

lished) that the solutions of krait venom, when stored at 4°C are perfectly stable over periods upto 2 years. However the other venoms are much less stable in aqueous solution, which, in case of viperine venoms is probably because of their high proteinase activity.

One point which must be mentioned about this study is that since the snakes whose venom was studied were maintained in artificial environment in cages, no distinct seasonal variation could be noted. No attempt was also made to correlate venom toxicity with the age, sex or the nutritional status of the snakes.

The working for a standard elapid venom preparation may not be difficult, but the same cannot be said about viperine venoms. Special precautions must be taken to ensure that the potency of the viperine venom is not lost. It should be a regular practice to centrifuge the venom samples collected during a day, distribute the supernatant into vials immediately. The vials should then be frozen at - 40°C and stored at that temperature until lyophilization. The lyophilized, dry powder may thereafter be stored at temperatures between 4-10°C. This would probably lead to lesser variation in the potencies of various batches of venom and pave the way for the setting up of a national standard for viperine venoms.

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OPTIMAL IN VITRO CONDITIONS FOR CONJUGAL TRANSFER OF R-PLASMIDS FROM *ESCHERICHIA COLI* TO *SALMONELLA TYPHI*

V. M. RANGNEKAR, S. V. GADRE, S. MUKERJI AND D. S. CHITNIS
Haffkine Institute, Parel, Bombay 400 012.

Our studies with *E. coli*-*S. typhi* mating system indicate that late logarithmic phase R^+ *E. coli* cultures mixed with R^- *S. typhi* in the ratio of 1 : 2 are optimal for conjugal transfer of R-plasmids.

INTRODUCTION

Two basic features of R-plasmids are their ability to replicate independently of the host chromosome (autonomous replication) and to effect their own transfer to other bacterial cells during conjugation (autotransferability) (1,2,3,4). The *in vitro* conjugal transmission of an R^+ culture is influenced by the physiology of the donor and recipient cells in the mating mixture which, in turn, is dependent upon the type of donor and recipient strain, donor-recipient cell density, possession of ordinary pili (type 1 pili) by the cells and environmental factors such as nutrients, pH, oxygen tension, temperature and presence of antibacterial agents (3,5,6). In conjugation studies, it is therefore, essential to optimize the *in vitro* conditions for a given mating system.

In this communication, we report the optimal growth phase of the donor culture and donor-recipient cell proportion for conjugal transmission of R-plasmids from *E. coli* to *S. typhi*.

MATERIALS AND METHODS

Donor strains — *E. coli* K12 F^- Lac $^+$ strains bearing various R-plasmids were kindly provided by Dr. E. M. Lederberg, Plasmid Reference Centre, California. Other R^+ *E. coli* strains were isolated from the faeces of individuals in Bombay.

Antibiotic sensitivity test — The antibiogram of each strain was determined by the method of Bauer *et al* (7). Antibiotic discs of known potency were placed on the surface culture of the test strain on Mueller Hinton agar medium and after 24 hours incubation, the plates were examined for zone of inhibition around each disc.

Selective media — Donor and transconjugant cells were selected on nutrient agar containing appropriate antibiotics shown below:

R-plasmids in <i>E. coli</i>	Drugs for selecting donor	Drugs for selecting transconjugants	Ref.
R 386	Tc	Tc,Nal	8
R 1	Cm,Ap	Cm,Ap,Nal	9
R 55	Cm,Ap,Km	Cm,Ap,Km,Nal	10
N 3	Sm,Tc	Sm,Tc,Nal	11
HIR 50	Cm,Ap,Tc	Cm,Ap,Tc,Nal	*
HIR 125	Cm,Ap,Tc	Cm,Ap,Tc,Nal	*

Ap — 10 mcg/ml ampicillin

Cm — 30 mcg/ml chloramphenicol

Km — 30 mcg/ml kanamycin

Nal — 60 mcg/ml nalidixic acid

Sm — 10 mcg/ml streptomycin

Tc — 30 mcg/ml tetracycline

* R-plasmids from faecal *E. coli* isolates at our laboratory.

RESULTS AND DISCUSSION

Determination of optimal growth phase of donor cells — Overnight nutrient broth (NB) cultures of donor and recipient strains were subcultured in fresh nutrient broth using 22% inoculum, and incubated at 37°C. Samples of the donor and recipient cultures were then withdrawn at different time intervals (Table I) and mixed in 5 ml nutrient broth. The organisms were allowed to conjugate at 37°C for one hour. The mixture was then vigorously agitated to separate the mating pairs, and 0.05 ml and 0.1 ml

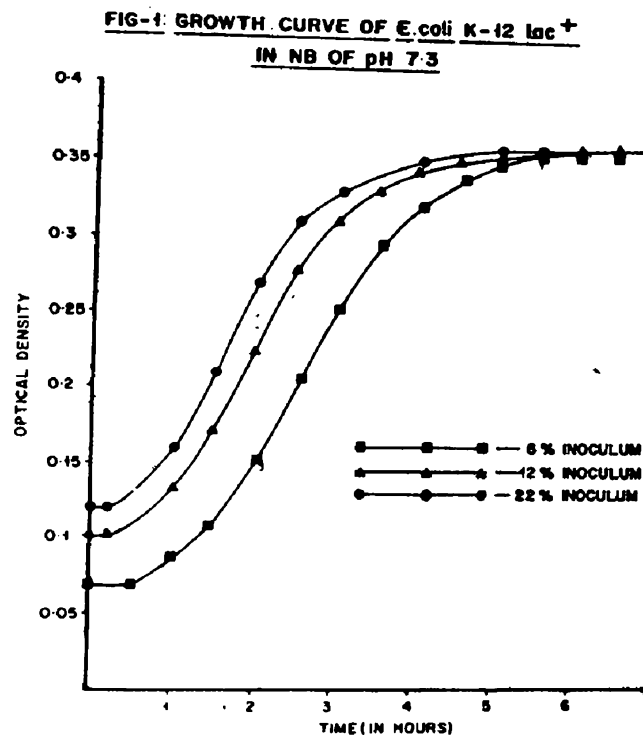
aliquots were plated on nutrient agar plates containing donor drug markers plus nalidixic acid for selecting transconjugants; 0.05 and 0.1 ml aliquots of 1:10,000 dilution of the mixture in physiological saline were plated on nutrient agar plates containing donor drug markers for selecting the donor cells. After incubation, the transconjugant and donor cell counts were taken and the conjugal transfer frequency was expressed as the ratio of transconjugants to donors in the mating mixture after one hour incubation at 37°C.

TABLE I

Conjugal transferability of R-plasmids using donor culture in different growth phases

Age (hrs.) of donor culture	Conjugal transfer frequency of R-plasmid (mean of 3 experiments)					
	R 386	R1	R 55	N3	HIR50	HIR125
2.0	3.1×10^{-3}	1.4×10^{-4}	2.4×10^{-4}	5.9×10^{-5}	0.8×10^{-6}	4.9×10^{-5}
3.0	4.0×10^{-3}	2.4×10^{-4}	2.7×10^{-4}	6.1×10^{-5}	8.8×10^{-6}	5.9×10^{-5}
3.5	4.3×10^{-3}	2.7×10^{-4}	2.8×10^{-4}	6.1×10^{-5}	9.1×10^{-6}	6.2×10^{-5}
4.0	4.4×10^{-3}	2.8×10^{-4}	2.4×10^{-4}	5.8×10^{-5}	9.1×10^{-6}	6.2×10^{-5}
4.5	4.1×10^{-3}	2.6×10^{-4}	2.1×10^{-4}	5.5×10^{-5}	6.0×10^{-6}	6.0×10^{-5}
5.0	3.6×10^{-3}	2.1×10^{-4}	1.8×10^{-4}	5.0×10^{-5}	5.3×10^{-6}	5.2×10^{-5}

Donor recipient proportion 1 : 2



Maximum transferability of R-plasmids to *S. typhi* was observed when late logarithmic phase cultures (3.0 — 4.5 hrs old) were employed as donors (Fig. 1 and Table I). The results have been

analysed statistically and the transfer frequencies with 2.0 hrs and 5.0 hrs cultures were found to differ significantly ($P < 0.05$) from the transfer frequency values with late log phase cultures.

In the above experiments, however, an inoculum size of 22% was used to prepare the late logarithmic phase cultures. To check whether the inoculum size employed influences the transfer frequency, late logarithmic phase cultures were prepared with 1%, 6%, 12% and 22% inoculum. The growth curves with 6%, 12% and 22% inoculum are shown in Fig. 1. With 1% inoculum the log phase started after 8½ hrs and lasted up-

to 21 hrs. The slope of all the curves was the same. The results presented in Table II indicate that nearly the same transfer frequency was obtained irrespective of the inoculum size used to prepare the donor cultures. As 22% inoculum gave donor cells with maximum competency in a short period of 4 hrs, this 'inoculum size' was used in subsequent experiments.

TABLE II

Conjugal transferability of R-plasmids with late logarithmic phase donor cultures prepared from different inoculum sizes.

Inoculum size	Conjugal transfer frequency of R-plasmid (mean of three experiments)	
	R 1	R 55
1 %	2.5×10^{-4}	2.6×10^{-4}
6 %	2.6×10^{-4}	2.7×10^{-4}
12 %	2.6×10^{-4}	2.9×10^{-4}
22 %	2.8×10^{-4}	2.8×10^{-4}

Late exponential phase cultures have been demonstrated to be highly competent donors of R-plasmids, also, by Harada and Mitsuhashi (5), and Anderson (6). The donor competence of an R⁺ cell population is dependent upon the proportion of cells with R-pili (12). In wild type R-plasmids the pilus synthesis is self-repressed and hence their transfer frequency is usually low. However, a transient derepressed state may follow during late logarithmic phase of the R⁺ culture during which pilus synthesis is fully expressed

and genetic transfer is maximum (6).

Optimal donor-recipient proportion — To determine the optimal donor and recipient proportion for the conjugal transfer of R-plasmids, conjugation experiments were conducted with different donor-recipient cell ratios. The donor and recipient cell counts of the late log phase cultures were predetermined. Aliquots of donor, and 0.5 ml of the recipient culture were introduced into 5 ml nutrient broth so as to obtain donor-recipient cell ratios of 1:8, 1:2

and 1:1 with the number of recipient cells remaining constant. The results are presented in Table III. It was observed that the

transfer frequency of the R-plasmids remained fairly constant in the range of donor-recipient ratios tried.

TABLE III

Conjugal transferability of R-plasmids using different donor-recipient proportions in the mating mixture

Donor-recipient proportion	Conjugal transfer frequency of R-plasmid (mean of 3 experiments)					
	R 386	R1	R55	N3	HIR50	HIR125
1:8	4.2×10^{-3}	2.6×10^{-4}	2.6×10^{-4}	6.3×10^{-3}	9.2×10^{-6}	6.1×10^{-5}
1:2	4.3×10^{-3}	2.6×10^{-4}	2.6×10^{-4}	6.3×10^{-3}	9.0×10^{-6}	6.2×10^{-5}
1:1	4.1×10^{-3}	2.3×10^{-4}	2.4×10^{-4}	6.1×10^{-3}	9.1×10^{-6}	5.9×10^{-5}

Earlier investigators (6,13,14,15) have reported high transfer frequencies of R-plasmids in *E. coli* x *E. coli*, *E. coli* x *Shigella* and *E. coli* x *S. typhimurium* crosses by employing lower proportion of donor cells in the mating mixture. In the present studies involving *E. coli* x *S. typhi* crosses, however, the frequency of R-plasmid transfer did not seem to be influenced by the donor cell proportions employed. It is possible that although an increase in the donor cell proportion may well in-

crease the frequency of initial R-factor transfer from *E. coli* to *S. typhi*, the frequency of subsequent R-factor transfer from *S. typhi* may be very low, thus diluting out the initial effect of increased donor cell proportion.

ACKNOWLEDGEMENT

The authors are grateful to Dr. B. B. Gaitonde, Ex-Director, Haffkine Institute for providing excellent facilities to carry out the present study.

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SYNTHESIS AND STUDY OF THIOUREA, N-(SUBSTITUTED ARYL (OR ALKYL)-N'[6-(2,4-DIAMINO) QUINAZOLINYL] AS POSSIBLE ANTITUBERCULOSIS AGENTS : II

B. G. KHADSE, S. R. LOKHANDE, MADHUBEN PATEL R. AND
V. L. KHAIRNAR

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

A series of N-(substituted-aryl or alkyl)-N'-[6-(2, 4-diamino) quinazolinyl] thioureas were synthesised by reacting 2, 4, 6-triaminoquinazoline with alkyl or substituted aryl isothiocyanates in boiling alcohol and tested for antituberculosis activity *in vitro* against *M. tuberculosis* (H₃₇Rv strain). Some of the compounds showed activity at 1.56 μ g/ml.

INTRODUCTION

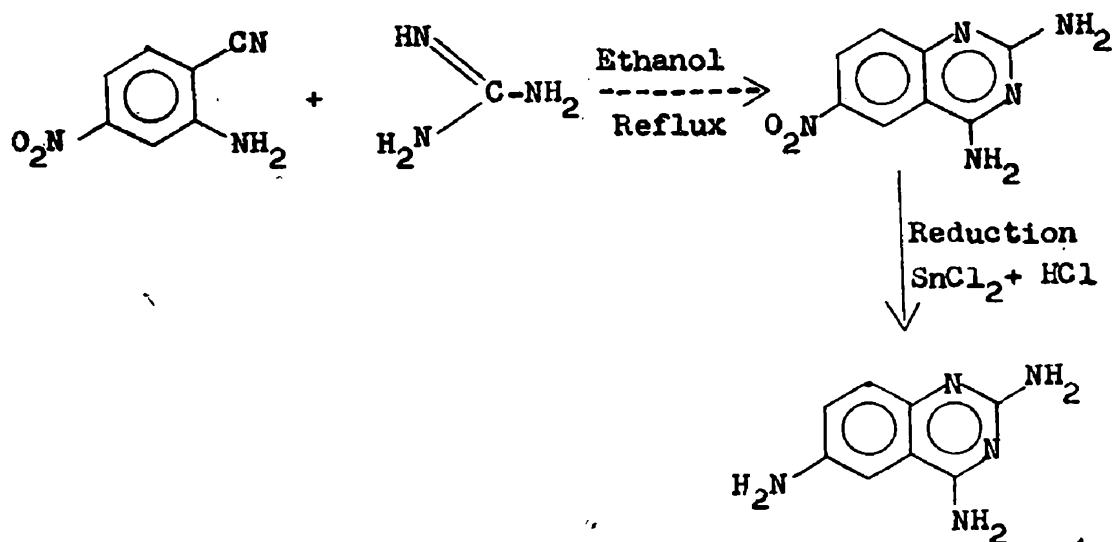
Initial studies by Elslager *et al.* (1) were oriented towards the synthesis of representative classical and non-classical analogs of folic acid. They reported various 2,4,6-triaminoquinazoline derivatives for their significant antibacterial and antimalarial activities (2). These encouraging results prompted the authors to prepare thiourea having amino quinazoline nucleus, as thioureas are also known to exhibit antituberculosis activity (3). With this in view, N-(substituted aryl or alkyl)-N'-[6-(2,4-diamino) quinazolinyl] thioureas have been prepared and described in this communication.

CHEMISTRY

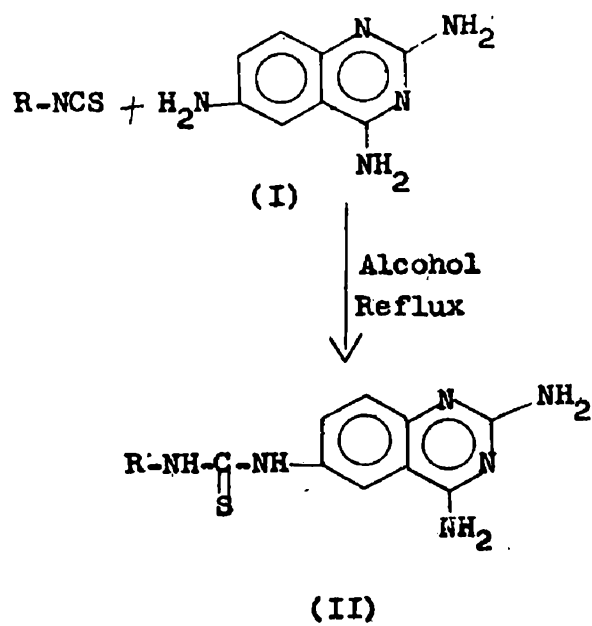
Since the only 6-amino group from 2,4,6-triaminoquinazoline (4) shows aromatic properties and is quite basic for further reaction with different alkyl or aryl isothiocyanates, 2,4,6-triaminoquinazoline was reacted in ethanol with isothiocyanates to obtain substituted thiourea derivatives (II) (Scheme-II). The requisite 2,4,6-triaminoquinazoline (I) intermediate was prepared according to the method of Davoll *et al.* (4) (Scheme-I).

The alkyl or substituted aryl isothiocyanates were prepared by known methods (5,6).

SCHEME - I



SCHEME - II



EXPERIMENTAL

The general method for the preparation of thiourea, N-(substituted-aryl or alkyl)-N'-[6-(2,4-diamino) quinazolinyl] is illustrated by the following typical experiment.

Thiourea, N-(4-bromophenyl)-N'-[6-(2,4-diamino)-quinazolinyl]:

A mixture of 0.350 g of 2,4,6-triamino-quinazoline (0.002 mole) and 0.428 g of 4-bromophenyl isothiocyanate (0.002 mole) was taken in 30 ml of ethanol. The reaction mixture was refluxed for 4 hours on steam bath. The solvent was then removed under reduced pressure to yield semicrystalline solid of the required thiourea derivative, (No. 11). This was recrystallised from methanol.

Yield: 0.520 g (66.8%); m.p. 180-82°C.

Similarly, all other compounds reported in Table I were prepared.

BIOLOGICAL RESULTS

A serial dilution method of Doub and Youman (7) was used to screen these compounds for *in vitro* antituberculosis activity against *M. tuberculosis* (H₃₇Rv strain).

Out of seventeen compounds, compound Nos. 15 and 17 inhibited the growth of *M. tuberculosis* at 1.56 µg/ml concentration. Compound Nos. 13 and 16 were active at 6.25 µg/ml. concentration and other compounds have the anti-tuberculosis activity at concentrations ranging from 12.5 to 100 µg/ml.

It was observed that N-substituted aromatic thioureas showed better activity than N-substituted aliphatic thioureas. However, N-alkoxy-phenyl derivatives were found to be more active than the corresponding N-substituted phenyl derivatives.

ACKNOWLEDGEMENT

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

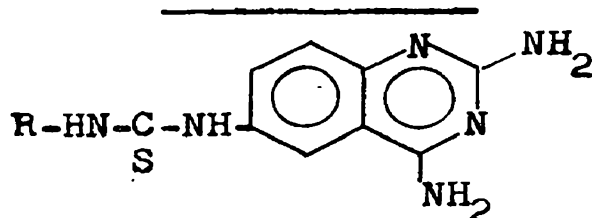
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TABLE I

Thiourea, N - (substituted - aryl or alkyl) -N'-[6-(2, 4-diamino)-quinazolinyl]



No.	R	Yield %	M.p. ^o C ^a (uncorr)	Mol. formula ^b	M I C ^c (μg/ml).
1.	CH ₃	68	245	C ₁₀ H ₁₂ N ₆ S	12.5
2.	C ₂ H ₅	86	212	C ₁₁ H ₁₄ N ₆ S	200
3.	n-C ₃ H ₇	78	208	C ₁₂ H ₁₆ N ₆ S.H ₂ O	100
4.	n-C ₄ H ₉	80	210	C ₁₃ H ₁₈ N ₆ S	50
5.	iso-C ₄ H ₉	56	212	C ₁₃ H ₁₈ N ₆ S	50
6.	Cyclo-C ₆ H ₁₁	72	201	C ₁₅ H ₁₉ N ₆ S	25
7.	Allyl	81	202	C ₁₂ H ₁₄ N ₆ S	50
8.	COOC ₂ H ₅	67	237	C ₁₄ H ₁₄ N ₆ O ₂ S	Inactive
9.	C ₆ H ₅	66	159	C ₁₅ H ₁₄ N ₆ S	100
10.	4-Cl.C ₆ H ₄	76	108	C ₁₅ H ₁₃ ClN ₆ S	25
11.	4-Br.C ₆ H ₄	67	182	C ₁₅ H ₁₃ BrN ₆ S	25
12.	4-F.C ₆ H ₄	82	185	C ₁₅ H ₁₃ FN ₆ S	50
13.	4-CH ₃ .C ₆ H ₄	64	176	C ₁₆ H ₁₆ N ₆ S	6.25
14.	4-CH ₃ O.C ₆ H ₄	62	192	C ₁₆ H ₁₆ N ₆ OS	25
15.	4-C ₂ H ₅ O.C ₆ H ₄	72	228	C ₁₇ H ₁₈ N ₆ OS	1.56
16.	4-C ₃ H ₇ O.C ₆ H ₄	79	175	C ₁₈ H ₂₀ N ₆ OS	6.25
17.	4-C ₄ H ₉ O.C ₆ H ₄	70	172	C ₁₉ H ₂₂ N ₆ OS	1.56

(a) Melting points are capillary m. p. and are uncorrected.

(b) All compounds analysed for C, H, N and found in the range $\pm 0.4\%$.

(c) Minimum inhibitory concentration for *in Vitro* antituberculosis activity (for INH = 0.04 μg/ml and for PAS = 0.4 μg/ml).

RESEARCH HIGHLIGHTS

Studies on the role of somatic antigens in the pathogenesis of cholera.

The department of Clinical Pathology has been working on the mechanism of protection afforded by conventional cholera vaccine. In their studies they have investigated the importance of the somatic antigens in adhesion of *V. cholerae* to the intestinal epithelium. It has been observed that antisera prepared against somatic antigens, protect against challenge with live *V. cholerae* in rabbit ileal loop model and this protection disappears when the antibodies are absorbed from the serum. While studying the mechanism of protection by this antiserum, proliferation and toxin elaboration by bacterial cells was found to be unaffected, but there was a significant inhibition in adhesion of the bacterial cells to the ileal epithelium.

Further both antisomatic antibodies and pure LPS of *V. cholerae* inhibited in vitro adhesion of

V. cholerae to the intestinal epithelium. Quantitation of the minimum amount of toxin required for fluid accumulation revealed that, adherant organisms could induce fluid secretion with smaller amounts of toxin, in comparison with in vitro prepared toxin deposited in the lumen for fluid induction. The data thus obtained supports the importance of adhesion of the organisms to the ileal epithelium. There is a long felt need for a safer and longer acting vaccine for the immunoprophylaxis of cholera. It appears that antigens used in the conventional vaccines are not improper as far as the protection is concerned but the difficulty lies in achieving sustained effective concentration of antibodies at the site.

R. B. Ghooi
Scientific Secretary

BOOK-REVIEW

Title : Vision in Invertebrates A — Invertebrate Photoreceptors

Editor : Hansjochem Autrum 729 pages, 314 figures, 1979

Cloth ISBN-3-50-08837-7

Springer Verlag, Berlin Heidelberg New York

Since the discovery of Vitamin A in the corneal tissue and the study of its function by Wald and his co-workers, there has been a spurt in the field of vision research. For a scientist in this field it is increasingly difficult to go through the new literature appearing periodically. Autrum has performed a yeoman service in this field by taking up the task of compiling the available data on invertebrate vision, and this book covers only a part of the subject by focussing only on Invertebrate Photoreceptors.

Photoreceptors are by definition the sites of transduction of light impulses in electrical potentials which can be readily transmitted and translated at higher centres. They resemble in function the other physiological receptors varying only in the form of their excitation energy. Photoreceptors are however more understood and accurately pinpointed, which cannot be said of other receptors.

Compiling data on invertebrate photoreceptors, their structure,

biochemistry, biophysics, and genetic dependance serve the function of elucidating the mechanisms of vision. More important is the impetus given to the understanding of the evolutionary mechanisms, and developments of visual mutants. Such data also aids the understanding of adaptation of special senses depending on environment, and circadian rhythms. The first chapter of the book by B. Diehn is devoted to photic responses and sensory transduction in Protists. It has been known for more than 150 years that a number of unicellular organisms respond to light stimuli by aggregating to either the lighted or darkened sites. These organisms not only can sense light but can also determine the direction of light. In this chapter Diehn has reported the work done on the molecular basis of the photo-sensory response/stimulus system, and the interactions of the photo-sensory and energy transducing systems.

Ocular Optical Filtering forms the subject of the second chapter

of the book by W. H. Miller. The author discusses the function of the various components, with special reference to the various filters for eliminating reflected, refracted light in invertebrates. These mechanisms are suggested to modulate the information passed on to the nervous system, and to convey to the higher centres an integrated representation of the photic responses.

Visual pigments are discussed by K. Hamdorf in the next chapter. The pigment biochemistry and the changes taking place in it are discussed with reference to the various classes of invertebrates. The physics of the compound eyes is discussed by A. W. Synder in the chapter immediately proceeding this, followed by reviews on photoreceptor potentials, pseudo-pupils, superposition of eyes, and colour vision.

An interesting chapter is that of Heisenberg on the genetic approach to visual studies. It was only in 1967 that the first visual mutant in *Drosophila* was reported. Since then attempts have been made to study various mutants for differences in photoreception, visual pigments and selective changes in spectral sensitivity of mutant retinal cells. *Drosophila* are being actively studied for mutants which would hopefully give a clue to the genetic dependence of visual system. This field is relatively new and much work needs to be done before any definite statement can be made on genetic visual disturbances in man.

The handbook is of great importance to workers in the field of vision, it is well illustrated and gives exhaustive information on the topics chosen by the Editor.

R. B. Ghooi
Scientific Secretary

PROCEEDINGS OF 'THE CRITICS' CLUB

(April to December 1979)

Dr. M. M. Storey of the Department of Biology, University of Salford, UK, spoke before the Critics' Club on the 9th of April 1979. In a well illustrated talk he described the work of the department on the effects of nutritional status on host parasite relationships in cotton rat filariasis. The talk proved to be useful to a large number of the Institute staff who are working on the problem of filariasis. In the lively discussion which followed, a large number of problems were discussed which would be of benefit in understanding the characteristics of the disease.

Shri H. S. Ved of the Department of Biochemistry next addressed the Club on the effects of dietary calcium and Vitamin D on lipid metabolism. After studying the effects of Vit. D in presence of high and low calcium diets he concluded that the vitamin induced changes in the lipid metabolism through its active metabolite known as 1,25-(OH)₂D₃.

The next meeting of the Club was addressed by Dr. M. J. Shied of the Middlesex Hospital and Medical School, of U.K. Dr. Shield has conducted a number of surveys on the environmental mycobacteria and their relation to the prevalence of Leprosy. He spoke of his work done in various areas of Africa and defined the relations between the incidence of *M. leprae* and other mycobacteria.

Shri K. S. Pradhan of the Department of Pharmacology of *leprae* and other mycobacteria.

on the 11th of May, his talk was on the problems of drug resistance in Maharashtra. The Department of Pharmacology has a sponsored project to study the incidence of drug resistance to Dapsone, which is a major contributory factor for the failure of treatment of leprosy. The cases of leprosy which were reported to be resistant to Dapsone were used for the study. The organisms obtained from them were injected in the mouse foot pad and these animals were tested for Dapsone resistance. The speaker revealed that of 150 suspected cases, 47 were biopsied and 17 were found to be totally resistant. Of the rest, 16 showed partial resistance.

Ms. S. W. Awchat, of the Department of Immunology, spoke before the Club on the 18th of May. Ms. Awchat has been working on the purification of mycobacterial antigens which induce delayed hypersensitivity reactions. She revealed that a number of fractions obtained from Purified Protein Derivative of the H₃₇ RV strain of human tuberculosis have shown activity when tested on known cases of tuberculosis.

On the 8th of June Shri A. P. Kulkarni of the Virology department addressed the Club. He spoke of his work on the markers of viral hepatitis viz., Serum Glutamic oxaloacetic and pyruvic transaminase levels. HIBOMA and human hepatitis B virus on injection in rabbits caused significant rise of the biochemical markers. Results of the three year study were discussed by him.

Shri V. M. Rangnekar of the Department of Bacteriology addressed the Club on the 29th of June. His work on conjugal transferability and genetic stability of R-plasmids revealed that this transfer of plasmids could contribute to the spread of drug resistance in diseases like typhoid.

Dr. R. N. Pithappan of the Department of Immunology Madurai University addressed the Club on 17th of July 1979. He discussed his work on T and B cells in the lizard. At the next meeting on 21st of August Dr. Gokak on human memory. Based on available literature and his work he propounded the hypothesis that "try not to remember in order to remember a thing".

Ms. Mangal Vaidya of the Blood Bank of the Institute addressed the Critics' Club on 5th October. The theme of her talk was red cell enzymes in haematological disorders. During the study, 210 cases with disorders and 100 normal individuals were studied. The levels of Glutathione peroxidase and Catalase in leukemias and various anaemias were discussed.

Studies on snake repellents, was the topic of the talk delivered by

Shri N. K. Sutar, of the Zoonoses Department on December 27. The Ministry of Defence, Government of India was interested in the development of effective snake repellants for use in forward areas, and has sponsored a scheme for the isolation and studies of snake repellents. Shri Sutar first described the various techniques used by him to assay the potential repellents and then went on to discuss their effects. The oil of *Acorus calamus* has shown promising activity, and further studies are in progress, he said.

At the last meeting for the year 1979, the Critics' Club was addressed by Professor Anthony T. Tu, of the Department of Biochemistry of the Colorado University. Prof. Tu is a well known authority on snake venoms and has greatly added to our knowledge on venom biochemistry. He discussed the effects of various fractions of different venoms, and their biochemistry. His talk was followed by a film show on sea snakes.

R. B. Ghooi
Scientific Secretary.

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DIRECTIONS TO CONTRIBUTORS

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 :

- (i) Reuben, R.; Carey, D. E. and Meyers, R. M. (1968)—Japanese encephalitis studies in Vellore, S. India, *Indian J. Med. Res.*, **56**, 109-18
- (ii) Cruickshank, R. (1968)—Medical Microbiology, 11th Ed. 180-4, E and S Livingstone Ltd., Edinburgh, p. 28.

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