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Haffkine Institute for Training, Research and Testing
Parel, Bombay 400 012, India

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Dr. Nirmal Kumar Dutta



1913 – 1982

OBITUARY

In the death of Dr. N. K. Dutta, on 2nd May, 1982, the country has lost a distinguished son. The loss will be deeply felt by the staff of Haffkine Institute whose Director he was, by the biomedical scientists to whose fraternity he belonged, and by the common man towards whose good he worked.

Dr. Nirmal Kumar Dutta, son of Dr. Asutosh Dutta, the then Civil Surgeon, was born on 1st December 1913, in Calcutta. His early education was completed at Dilajpur Lilla School, and he graduated in Medicine from the Carmichael Medical College, Calcutta, in 1937. After his graduation he spent a few years as a pharmacologist at the Central Drugs Laboratory, Calcutta. He also spent some time at the School of Tropical Medicine, Calcutta. In 1947 he left for Oxford, where he obtained his D. Phil.

Dr. Dutta was appointed the Assistant Director in the Department of Pharmacology at this Institute in 1949 a post he held till his appointment as Director in April 1968. Dr. Dutta headed the Institute till December 1971, when he retired to take over as the Deputy Director, Indian Council of Medical Research (ICMR). After leaving ICMR Dr. Dutta was for a short time associated with the Vaccine Institute and later with Bhailal Amin Hospital in Baroda. He left Baroda in 1980 to live and work in Calcutta.

Dr. Dutta's early work was on classical pharmacology, and indigenous drugs. In 1955 he established the infant rabbit model for the evaluation of drugs against cholera. With this model began the most important phase of Dr. Dutta's research career. He published as many as 27 papers in journals of international repute on cholera. His authority on the subject was such that the World Health Organisation appointed him on the Expert Advisory Panel on cholera in 1967.

Throughout his life Dr. Dutta's industry was recognised and appreciated. He won a large number of scholarships and awards, important among which were the K. K. Mukerjee Scholarship (1944-46), the Vincent Massey Fellowship, Canada, (1944-45), Rockefeller Foundation Grant (1962-63), U.S. Army Grant (1962-63), the Basanti Devi Amichand Award (1955) and the G. J. Watermull Award (1955). He was also made a fellow of various prestigious bodies like the National Institute of Sciences and the Academy of Medical Sciences. In 1964 he was honoured by Oxford University with a D.Sc. in recognition of his studies on cholera.

As an administrator Dr. Dutta combined strictness with justice and earned the respect of every member of the staff. He had great love for cleanliness and an eye for natural beauty, and it was during his time that the entire institute got a face lift.

Dr. Dutta is no more, but his memory will always encourage those who strive for scientific knowledge and perfection. May his soul rest in peace!

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Antibacterial Activity of *Semecarpus anacardium* Extracts

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and K. D. SHARMA

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Semecarpus anacardium Linn. is widely used in Ayurveda for various disorders. It has been reported to possess *inter alia* anti-tumor and antiinflammatory properties. Extracts of pericarp oil were tested against various bacteria and were found to possess potent inhibitory activity against *Clostridium tetani* and *Clostridium welchii*. The extracts were found to be inactive against all other aerobic bacteria tested *in vitro*.

Semecarpus anacardium is a widely growing tree distributed mainly in the tropical countries. Since many years it has served as a home remedy for a number of disorders and has been used by Ayurvedic physicians.¹ Among the clinical uses of the nut are anti-inflammatory, antibacterial and anthelmintic.² Chemical investigations on the oil have demonstrated the presence of two components 1, 2 dihydroxy 3-pentadeca-7'-10' dienyl benzene and 1, 2 dihydroxy 3-pentadeca 8' -enyl benzene i.e. diene and monoene bhillwanols respectively.³ These two components form the bulk of the pericarp oil constituting about 90 per cent of the content. Recent reports have demonstrated that the oil possesses significant antitumor activity against 9 KBN carcinoma,⁴ Yoshida sarcoma, Daltons lymphoma, mouse-fibrosarcoma and even against human epidermal carcinoma.⁵ The oil is highly vesicant and causes disruption of mast cells leading to massive histamine release. The oil is also report-

ed to have nonspecific smooth muscle relaxant properties.⁶

We have studied the extracts of the pericarp oil and observed their ability to inhibit the growth of *Cl. tetani* (N.S.) Harvard strain, *Cl. tetani* sporulating A-471 and *Cl. welchii* B-58-1. The extracts were found to be inactive against *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Bacillus megaterium*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Vibrio cholerae*, *Shigella shigae*, at the concentration of 100 μ g/ml of the extracts.

Extraction : The crude oil was obtained by crushing the nuts and extracting with chloroform by cold percolation. The solvent was evaporated completely under reduced pressure to get dark brown oil. This crude oil was distilled under reduced pressure to get a light yellow coloured fraction mostly consisting mono-ene and di-ene bhillwanols.

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TABLE 1
EFFECT OF CRUDE AND DISTILLED CHLOROFORM EXTRACT ON THE
GROWTH OF BACTERIA AT THE CONCENTRATION 100 μ g/ml.

Bacterias	Growth after 24 hrs. incubation
1. <i>Escherichia coli</i>	+
2. <i>Proteus vulgaris</i>	+
3. <i>Staphylococcus aureus</i>	+
4. <i>Bacillus megaterium</i>	+
5. <i>Bacillus subtilis</i>	+
6. <i>Pseudomonas aeruginosa</i>	+
7. <i>Serratia marcescens</i>	+
8. <i>Vibrio cholerae</i>	+
9. <i>Shigella shigae</i>	+
10. <i>Clostridium tetani</i> (N.S.) Harvard strain	—
11. <i>Clostridium tetani</i> A-471	—
12. <i>Clostridium welchii</i> B-58-1	—

Key: + = Growth.
 — = No growth.

The fractions were dissolved in ethylene glycol.

Organisms used :

Cl. tetani (Non-sporulating) Harvard strain *Cl. tetani* A-471, *Cl. welchii* B-58-1.

Inoculum used :

24 hour old culture adjusted to 50% transmission at 640 nm.

Medium :

For aerobes-Respective nutrient broth.
 For anaerobes-Thioglycollate broth (Difco).

Antibacterial activity was initially tested with 100 μ g/ml concentration of the

extracts against all the bacterias mentioned.

Set I: For the determination of minimum inhibitory concentration (MIC), different concentrations of fractions were prepared in sterile ethylene glycol from 100 μ g/ml to 3.312 μ g/ml. Medium used for the assay procedure contained 4.5 ml sterile thioglycollate broth per tube. 0.1 ml of the inoculum was aseptically transferred to each of the tube followed by the addition of 0.5 ml of the prepared concentration of fractions. All the tubes were incubated for 24 hours at 37°C.

Set II: To find out whether the inhibitory effect is of bacteriostatic type or

TABLE 2

Test tube No.	Concentration μ g/ml.	Growth observed					
		<i>Cl. tetani</i> (N.S.) Harvard		<i>Cl. tetani</i> A-471		<i>Cl. Welchii</i> B-58-1	
		C	D	C	D	C	D
1.	20	—	—	—	—	—	—
2.	10	—	—	+	—	+	—
3.	5	—	—	+	+	+	+
4.	2.5	+	—	+	+	+	+
5.	1.25	+	+	+	+	+	+
6.	0.625	+	+	+	+	+	+
7.	0.312	+	+	+	+	+	+
8.	Control 1	—	—	—	—	—	—
9.	Control 2	+	+	+	+	+	+
10.	Control 3	+	+	+	+	+	+

Key : + = Growth. — = No growth.

D = Distilled chloroform extract. C = Crude chloroform extract.

Control 1 = Thioglycollate broth 4.5 ml. + 0.5 ml. distilled water.

Control 2 = Thioglycollate broth 4.5 ml. + 0.5 ml. distilled water.
+ 0.1 ml. inoculum.

Control 3 = Thioglycollate broth 4.5 ml. + 0.5 ml. ethylene glycol.
+ 0.1 ml. inoculum.

bacteriocidal type, 0.1 ml from each of the tubes of set I was used as an inoculum and was transferred aseptically to set of tubes containing 5 ml sterile thioglycollate broth, so as to lower down the concentration of fractions far below their respective MIC values. All the tubes from the set were further incubated for 24 hours at 37°C.

The crude chloroform extract as well as distilled chloroform extract were found to be inactive against all the aerobic species tested (Table 1.), while both

the extracts showed inhibition against *Cl. tetani* and *Cl. welchii*. So an attempt was made to find out their MIC.

MIC of the crude oil was found to be 5 μ g/ml, 20 μ g/ml, 20 μ g/ml for *Cl. tetani* (N.S.) Harvard strain, *Cl. tetani* A-471, and *Cl. welchii* B-58-1 respectively. There was significant increase in the activity when the crude fraction was distilled. The distilled fraction was found to contain mostly two components monoene and diene bhlwanols and its MIC was found to be 2.5 μ g/ml 10 μ g/ml,

10 μ g/ml for *Cl. tetani* (N.S.) Harvard strain, *Cl. tetani* A-471 and *Cl. welchii* B-58-1 respectively (Table 2).

Even after lowering the concentration of fractions below their respective MIC values, growth pattern observed in all the tubes was identical on reinoculation, indicating the action to be bacteriocidal. Also, looking at the nature of the components present in the distilled fraction i.e. monoene and diene bhlwanols, the

activity may decrease on preserving the extracts because of complex oxidation process.

Acknowledgement

We thank the Director, Haffkine Institute, Bombay and Dr. John Barnabas, F.N.A., F.A.Sc., Professor and Head, Post Graduate School for Biological studies, Ahmednagar College, Ahmednagar, for his guidance and keen interest in this work.

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Studies on Anaphylaxis III-Phospholipase A₂ Induced Mouse Hind Paw Oedema

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Phospholipase A₂ enzyme (EC 3.1.1.4) injected in the mouse hind paw was found to produce an oedema with two peaks. The oedema curve was compared with that of compound 48/80. Studies conducted using antagonists of autocooids and an inhibitor of prostaglandin, demonstrate that the oedema is mediated by histamine and prostaglandins. Slow reacting substance of anaphylaxis and 5—hydroxytryptamine do not appear to be involved in the reaction.

Introduction

Feldberg and Kellaway (1938) and Feldberg *et al* (1938) demonstrated that cobra venom could induce the release of histamine and the formation of lysolecithin along with that of an unidentified smooth muscle stimulating substance. Subsequent studies suggested that the phospholipase of the snake venom was responsible for these effects of the venom; (Orange and Austen, 1969; Piper and Vane, 1971; Moncada and Vane, 1979; Burka and Flower 1979).

Blackwell *et al* (1978) demonstrated an increase in phospholipase A₂ (Ph A₂) activity in sensitized lung on challenge with antigen thus involving Ph A₂ in antigen induced mediator release. Nakao *et al* (1980) demonstrated that Ph A₂ is involved in A 23187 (a calcium ionophore) induced histamine release from rat mast cells.

Selye (1937), first suggested that a number of substances capable of liberat-

ing biologically active agents induced reactions which are similar to anaphylactic reactions. It was demonstrated that egg white, hyalurodinase and dextran could induce an anaphylactoid oedema in the rat paw which could be easily quantitated. Further studies suggested that the reactions were mediated by histamine (Halpern and Briot, 1952) and 5 hydroxy tryptamine (5-HT) (Halpern *et al* 1957).

Ph A₂ has been demonstrated to cause a hydrolysis of the phospholipids to yield lysolecithin and free fatty acids, predominantly of an unsaturated type (Hanan; 1952). The free fatty acids can undergo cyclooxidation to yield prostaglandins (PGs) (Kunze and Vogt; 1971), alternatively they may undergo lipooxidation to form Slow Reacting Substance of Anaphylaxis (SRS-A) (Burka and Flower, 1979; Hitchcock and Kokolis, 1981). The lysophospholipid can be acetylated to yield the Platelet Activating Factor (PAF) (Cusak; 1980) which has

been suggested to be a mediator of anaphylaxis (Vargaftig *et al*, 1980).

Since Ph A₂ can release a host of substances which are mediators of anaphylaxis, it was of interest to study Ph A₂ induced anaphylactoid reactions in the foot pad of experimental animals.

Materials and Methods

All drugs and chemicals used, were of analytical or an equivalent grade. Ph A₂ enzyme was purchased from CSIR, Delhi, the activity of which corresponded to 1 mg. = 5 IU. The mouse hind paw thickness was measured as an index of oedema by Mitutoyo Pocket Thickness Gauge, Japan. Increase in paw thickness before and after intrapedal (i.p.d.) injection of Ph A₂ was expressed in units, each unit being equal to 0.001 inches.

For all the experiments the mice of Kasauli strain (either sex) weighing between 18 to 20 gms. supplied by Haffkine Bio-pharmaceuticals Corporation Limited were used. For each experimental set, six animals were employed and the results obtained were analyzed for statistical significance using Student's 't' test.

For the induction and measurement of an oedema the method described by Ghooi *et al* (1980) was used. Briefly the method is as follows: Hind paw thickness was measured prior to administration of Ph A₂, after which Ph A₂ was injected i.p.d. in the right paw in 0.05 ml. saline. The left paw received an equal volume of saline. An increase in the paw thickness in either paw was calculated by repeating measurements at fixed time intervals. Oedema was

expressed in units, each unit being = 0.001 inches.

For the studies on the involvement of the mediators, standard antagonists were injected intraperitoneally half an hour prior to administration of Ph A₂. On this model the following studies were conducted:

- (1) Time courses of the oedema formations by Ph A₂ and compound 48/80 a known histamine releaser.
- (2) Dose response curve of Ph A₂.
- (3) Effects of calcium chelation by ethylene diamine tetracetic acid (EDTA).
- (4) Effects of the antagonists of the mediators.
- (5) Effects of the steroidal antiinflammatory drugs (SAID) and the nonsteroidal anti-inflammatory drugs (NSAID).

Results

- (1) Time course of the oedema formations.

Ph A₂ at a dose level of 1.25 μ g in the paw produced an oedematous response with two peaks, the first appearing at 15-30 mins and second peak at 3-4 hours. Table (1) shows the course of the development of the oedema. On the other hand compound 48/80 (0.3 μ g/paw) produces a single peak at 10 mins. as seen in table (2).

- (2) Dose response curve.

In doses ranging from 0.3125 μ g to 2.5 μ g graded oedematous response was observed. Fig (1) shows the effects of these doses at different time intervals after injection of Ph A₂.

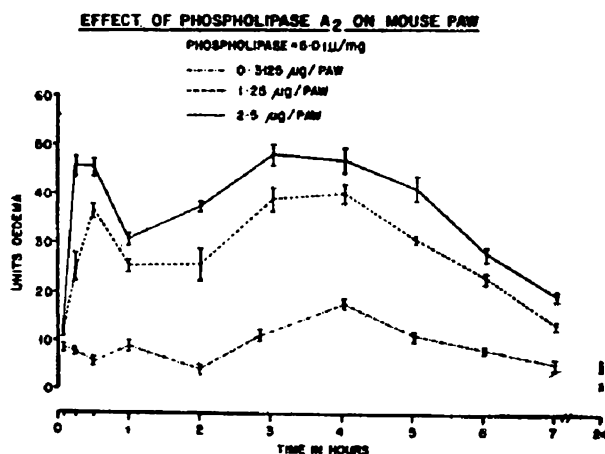


Fig. 1.

Table 1
Time course of oedema formation
of phospholipase A
(1.25 µg in 0.05 ml saline)

Time interval	Mean Oedema Units ± S.E.
5 min.	11.5 ± 0.96
15 min.	25.0 ± 2.9
30 min.	36.16 ± 1.7*
1 hr.	25.30 ± 1.36
2 hr.	25.83 ± 3.39
3 hr.	39.2 ± 2.86*
4 hr.	40.33 ± 1.80*
5 hr.	31.0 ± 0.63
6 hr.	23.16 ± 1.04
7 hr.	13.5 ± 0.88
24 hr.	1.0 ± 0.52

*Readings of Peak 1 & 2, significantly differ from 5 min. reading $P < 0.001$.

(3) Effects of EDTA.

Chelation of extra cellular calcium by EDTA at a concentration of 1 mM significantly inhibited both peaks of the oedema, though the oedema was not completely abolished. These results are shown in table (3).

(4) Effects of the standard antagonists.

Mepyramine, methysergide and atropine were tested, each at 1 mg./kg. dose

Table 2
Time course of oedema formation of
Compound 48/80
(0.3 µg in 0.05 ml saline)

Time interval	Mean Oedema Units ± S.E.
5 min.	8.5 ± 1.58
10 min.	24.0 ± 0.89*
30 min.	15.2 ± 0.00**
60 min.	8.4 ± 1.39
120 min.	3.2 ± 1.4

** $P < 0.001$

* $P < 0.05$

level. Mepyramine significantly inhibited the first peak of the oedema but was without any effect on second peak. Methysergide did not inhibit the either peak but was found to potentiate the second peak. Atropine produced partial inhibition of both the peaks. Table (4) shows the effects of these drugs.

(5) Effects of SAID & NSAID.

Indomethacin at doses 50, 100 and 200 mg./kg. produced significant inhibition of the first peak but was without effects on a second peak. However, there was no dose related inhibition seen. Table (5) shows the results of this test. Cortisone acetate (50 mg./kg.) inhibited both the peaks significantly. These results are seen in Table (6).

Discussion

Ph A₂ induces a dose related oedematous response with two peaks, the first appears at 30 mins and the second one appears at 3½ hours. This data viewed in the light of the release of the mediators induced by Ph A₂ (Feldberg & Kellaway, 1938; Nakao *et al* 1980) may be attributed to a similar mechanism in the paw.

Table 3
Effects of EDTA on Ph A₂ Oedema
 EDTA = 1 mM

Time	Mean Oedema Values			% Inhibition
	Control Units $\pm$ SE	Treated Units $\pm$ SE		
$\frac{1}{2}$ hr.	35.4 $\pm$ 1.568	27.0 $\pm$ 1.354*	21.46	
$3\frac{1}{2}$ hr.	41.6 $\pm$ 1.077	34.0 $\pm$ 1.378*	18.27	

*P < 0.01.

Table 4
Effects of antagonists of the mediators

Time interval	Antagonists	Mean Oedema Values					
		Control			Treated		
		Units	$\pm$	SE	Units	$\pm$	% Inhibition
$\frac{1}{2}$ hr.	Mepyramine maleate (1 mg/kg)	44.16	1.620		33.0	1.57*	25.2
$3\frac{1}{2}$ hr.	"	39.00	2.857		38.25	1.86**	1.9
$\frac{1}{2}$ hr.	Methysergide bimaleate (1 mg/kg)	43.16	1.36		46.2	1.20**	—
$3\frac{1}{2}$ hr.	"	37.00	2.352		53.0	1.953	—
$\frac{1}{2}$ hr.	Atropine (1 mg/kg)	45.33	2.22		36.66	1.66*	18.9
$3\frac{1}{2}$ hr.	"	34.00	0.733		25.20	1.80*	25.8

*P < 0.01

**N.S.

Table 5
Effects of NSAID on Ph A₂ Oedema

Time interval	Concentration of Indomethacin	Mean Oedema Values				% inhibition
		Control Units	SE	Treated Units	SE	
$\frac{1}{2}$ hr.	50 mg/kg	44.60	1.620	36.00	1.483**	19.2
$3\frac{1}{2}$ hr.	„	33.00	1.570	36.00	1.316*	—
$\frac{1}{2}$ hr.	100 mg/kg	43.66	1.360	36.00	1.580**	17.4
$3\frac{1}{2}$ hr.	„	37.00	2.350	37.25	2.280*	—
$\frac{1}{2}$ hr.	200 mg/kg	43.66	1.360	35.16	2.270**	19.4
$3\frac{1}{2}$ hr.	„	37.00	2.350	36.00	2.301*	2.7

*N.S.

**P < 0.01.

Table 6
Effects of SAID on Ph A₂ Oedema

Time interval	Concentration of Cortisone Acetate	Mean Oedema Values				% inhibition
		Control Units	± SE	Treated Units	± SE	
$\frac{1}{2}$ hr.	50 mg/kg	45.33	2.22	19.00	2.52**	58.00
$3\frac{1}{2}$ hr.	„	34.00	0.733	12.20	4.40**	64.10

* P < 0.001

Compound 48/80 also produces an oedematous response in the mouse hind paw which has a single peak appearing at 10 mins. The oedema has previously been described to be due to release of histamine but not that of 5-HT (Ghooi *et al* 1980). The patterns of oedema induced by Ph A₂ and by 48/80 suggest

that while both induce oedema through mediator release, the mediators in either case are probably different.

Studies with EDTA, demonstrated that both the peaks of oedema were sensitive to calcium deprivation caused by EDTA thus suggesting the role of calcium ions in Ph A₂ induced release of substances

from the cell. This observation is parallel to those made by Kunze and Vogt (1971)

The standard antihistaminic, mepyramine maleate caused a significant inhibition of the first peak of the oedema thus suggesting a role for histamine in the reaction but was without any effect on the second peak. This result suggests that mediators other than histamine are involved in the genesis of late response to Ph A₂. Atropine caused a significant inhibition of both the peaks of the oedema, revealing a role for endogenous acetylcholine in the response. However, methysergide bimalate failed to inhibit either of the peaks, thus suggesting that 5-HT has no role to play in the reaction.

NSAIDs are known to inhibit the formation of PGs while potentiating a formation of SRS-A. (Burka and Flower, 1979; Lakdawala et al 1981). Indomethacin on this model inhibited the first peak of oedema significantly. However, the inhibition was not dose related with respect to the doses of indomethacin and the inhibition was constant (at 17-19%) when indomethacin was used in doses ranging from 50 to 200 mg/kg. This result may be indicative of a small role of PGs in the genesis of the first peak. In the doses used, indomethacin failed to potentiate the second peak of an oedema, thus indicating the absence

of involvement of SRS-A in the second peak of oedema.

SAIDs have been observed to inhibit both the peaks of the oedema. These agents have been demonstrated to inhibit membrane disruption and exocytosis induced by various perturbing agents (Weissmann, 1973). Steroids thus protect the cells from the lytic activity of lysosomal hydrolases. It is probable that the same mechanism of action operates in the inhibition of Ph A₂ induced oedemas.

The aim of the study was to investigate the involvement of Ph A₂ in the mediator release, the present data suggests that Ph A₂ per se releases mediators. However it must be admitted that definitive proof of the role of Ph A₂ in the mediator release, induced by other agents is still far off. The results obtained however, are encouraging enough and efforts are going on to identify the various factors involved in the Ph A₂ induced anaphylactoid reaction.

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Failure of cholera toxin to induce a protective immune response against NAG vibrios

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Subcutaneous immunization of rabbits with pure cholera toxin protected them against live *Vibrio cholerae* Inaba and Ogawa strains and crude cholera toxin but not against three NAG vibrio strains, in the rabbit ileal loop model. The sera of these immunized rabbits possessed high cholera antitoxin activity. The present findings suggest that the toxin produced by at least some NAG vibrio strains may not be antigenically similar to cholera toxin.

Introduction

Non-agglutinating (NAG) vibrios have been shown to be responsible for a cholera-like disease in humans (1), and the enterotoxin produced by some NAG vibrios has been reported to be antigenically related to cholera toxin (2,3,4). Based on these observations, Finkelstein (5) speculated that an effective antitoxin immunity against cholera may provide a potentially significant fringe benefit against diarrhoea due to NAG vibrios. However, there is no experimental evidence indicating the plausibility of this speculation for NAG vibrio isolates. The present work was, therefore, undertaken to assess whether cholera toxin can induce protection against NAG vibrios.

Material and Methods

Vibrio cholerae 569B Inaba, *V. cholerae* 5321 Ogawa, NAG vibrio 280, NAG vibrio 281 and NAG vibrio 16 were obtained from the lyophilized culture

stock at Haffkine Institute, Bombay. Pure cholera toxin was kindly provided by Dr. R. A. Finkelstein. Crude cholera toxin was prepared from *V. cholerae* 569B Inaba by the method described by Hochstein *et al* (6).

Five Swiss albino rabbits weighing 1.5 kg. were immunized subcutaneously with 100 μ g of pure cholera toxin mixed with 1.0 ml. of Freund's complete adjuvant. The rabbits received the toxin in two divided doses at an interval of two weeks. Five rabbits in the control group received the same quantity of plain Freund's complete adjuvant at two weeks interval.

Antitoxin levels in the sera of immunized rabbits were determined two weeks following immunization, by passive haemagglutination (PHA) technique employing formalinized sheep red blood cells sensitized with crude cholera toxin as described by Hochstein *et al* (6).

Table 1

Cholera antitoxin levels and ileal loop response of immunized and control rabbits

Rabbit No.	Cholera anti-toxin titre ^c	Fluid accumulation (in ml/cm) in ileal loops challenged with					Crude cholera toxin ^d
		569 B Inaba	5321 Ogawa	NAG 280	NAG 281	NAG 16	
1 ^a	< 20	1.2	1.0	1.2	0.7	0.5	1.8
2 ^a	< 20	1.2	1.4	0.8	0.9	0.8	2.0
3 ^a	< 20	1.4	0.8	0.8	0.9	0.1	1.8
4 ^a	< 20	1.0	1.2	1.0	1.2	0.8	2.0
5 ^a	< 20	0.9	0.8	0.6	0.5	0.6	1.5
6 ^b	2560	0.2	0.0	1.0	0.8	0.5	0.0
7 ^b	10240	0.0	0.0	0.8	1.0	0.5	0.2
8 ^b	20480	0.0	0.0	0.8	1.0	0.9	0.0
9 ^b	5120	0.0	0.0	1.4	0.5	1.0	0.0
10 ^b	2560	0.0	0.0	1.2	1.0	0.8	0.2

Rabbit received plain Freund's complete adjuvant.

^b Rabbit immunized with 100 µg of pure cholera toxin along with Freund's complete adjuvant.

^c Cholera antitoxin titre determined by PHA test employing formalinized sheep red blood cells sensitized with crude cholera toxin.

^d Loops were injected with 1.0 ml. of 100 fold diluted crude cholera toxin.

The technique of De and Chatterjee (7) was employed for the challenge of immunized and control rabbits. The challenge dose consisted of a predetermined optimal concentration of 10⁶ live vibrio cells and crude cholera toxin (1 : 100 dilution) in separate loops, in duplicate, given two weeks after the second immunization dose.

Results

Two weeks following immunization, the antitoxin titres in immunized rabbits ranged from 2560 to 20480 (Table 1).

These rabbits did not accumulate fluid in their ileal loops when challenged with crude cholera toxin or with live *V. cholera* 569B Inaba and *V. cholera* 5321 Ogawa cells. However, loops challenged with NAG vibrios accumulated significant quantity of fluid which was comparable to the ileal loop response of non-immunized rabbits. Thus, rabbits immunized with cholera toxin were protected against crude cholera toxin and Inaba and Ogawa live vibrio cells but not against the NAG vibrios.

Discussion

The present data indicates that anti-toxic response against cholera toxin may not necessarily protect against all strains of NAG vibrios. This implies that cholera toxin and the toxin produced by at least some NAG vibrios may not be immunologically identical. Although, this appears contradictory to an earlier report (2) it should be realized that neither cholera toxin nor NAG vibrio toxin employed in the previous experiments (2) were of high purity and hence, cross reaction could be due to components other than the active toxin. It seems that this variation in observations may be basically due to differences in NAG vibrio strains employed.

Attempts to obtain even a crude preparation of NAG vibrio toxin at our laboratory proved futile, as the culture supernatants of the NAG vibrios used in the present study did not manifest enterotoxin activity in the rabbit ileal loop model. Chatterjee *et al* (8) too did not obtain enterotoxic activity in the culture supernatants of NAG vibrios, albeit in case of some NAG vibrios they could demonstrate permeability factor activity.

However, Ohashi *et al* (3) and Zunnaka and Carpenter (4) have reported *in vitro* enterotoxin production by the NAG vibrio strains used at their laboratory. It is apparent, therefore, that all NAG vibrios may not elaborate enterotoxin in conventional media such as the Syncase medium under the *in vitro* environmental condition. Surprisingly, however, data seems to be lacking on the confirmation of CAMP mediated action of NAG vibrio toxin, despite the availability of sensitive assay systems for the purpose. Thus, the possibility that the mechanism of action of the toxin elaborated by at least some vibrios not being CAMP mediated, has not been ruled out. Hence, further elaborate studies in this direction are essential for the understanding of the nature and mechanism of NAG vibrio toxin.

Acknowledgements

We thank Dr. R. A. Finkelstein, for the gift of pure cholera toxin. We are also grateful to the Director, Haffkine Institute, for the facilities to conduct these studies and for the permission to publish the data.

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Larvicidal Activity of *Lasiosiphon eriocephalus* (Dcne)*

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Lasiosiphon eriocephalus Dcne (Thymeleceae) Rametha is reported as a fish poison. This fact led us to investigate its toxicity to mosquito larvae (*Culex p. fatigans*). Petroleum ether extract of Rametha demonstrated very promising larvicidal activity at the concentration of 0.024%, with residual activity lasting upto 96 hours.

INTRODUCTION

It is a well known fact that plant based insecticides are less hazardous than synthetic ones. Large number of plants have been reported as having larvicidal and/or insecticidal properties in the literature.

Lasiosiphon eriocephalus Dcne (Fami— Thymeleceae) Rametha has been reported as fish poison (5). It is common on the hills of Western India. The bark is powerful vesicant and it is used to intoxicate fish. The leaves and bark are also used medicinally. The plant material was collected from Kas forest area, 20 miles away from Satara City in Maharashtra State. Earlier workers have reported phytochemical investigation but no insecticidal activity was seen (1-4).

MATERIALS AND METHODS:

The plant material was powdered to uniform mesh. One kg. dried powder

was extracted with petroleum ether (60-80°C) in Soxhlet for 48 hrs. The solvent was removed on water bath. Dried gummy extract (20 gms.) was taken for qualitative chemical analysis and mosquito larvicidal activity. Screening procedure was followed as per W.H.O. specification (6). Twenty-five — IV stage instar larvae were taken in 500 ml. beaker containing 250 ml. tap water. Crude petroleum ether extract was used in the concentration from 0.004 to 0.028%. Solvent control and water control was also taken simultaneously. Each experiment was carried out in triplicate.

RESULTS AND DISCUSSION

Table 1 shows larvicidal activity against mosquito larvae (*Culex-p-fatigans*) and residual activity. There was no mortality observed at 0.004% concentration. Hundred percent mosquito

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Table 1

Larvicidal activity of petroleum ether extract of *Lasiosiphon eriosephalus* (Dcne), Rametha.

Obs. No.	% concentration	% Mortality (24 hrs.)					
			48	72	96	120	144
1	0.004	Nil	—	—	—	—	—
2	0.008	04	—	—	—	—	—
3	0.012	24	—	—	—	—	—
4	0.016	64	—	—	—	—	—
5	0.020	80	—	—	—	—	—
6	0.024	100	100	100	100	72	60
7	0.028	100	100	100	100	100	100
8	Solvent control	Nil					
9	Water control	Nil					

Table 2

Late toxicity of petroleum ether extract of *Lasiosiphon eriocephalus* (Dcne), Rametha.

Obs. No.	% Concentration	% Mortality at hours			
		24	48	72	96
1	0.008	4	4	52	100
2	0.012	24	24	60	100
3	0.016	56	60	100	—
4	0.02	80	100	—	—
5	Solvent control	Nil	Nil	Pupae	Adults
6	Water control	Nil	Nil	Pupae	Adults

larvicidal activity was observed at the concentration of 0.024% within 24 hours. While studying the residual activity 100% mortality was noted upto 96 hours and 60% mortality at 144 hours with 0.024% concentration and with 0.028% concentration 100% mortality persisted for 144 hours.

It can be seen from table 2 that, with 0.008% to 0.020% concentration of petroleum ether extract of Rametha, arvae were not developed to pupae and adults and late toxicity was observed.

Based on the above observations,

isolation of active principles from *L. eriocephalus* responsible for the toxicity to culex larvae may be useful.

ACKNOWLEDGEMENT

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Book Review

Title : Topics in Environmental Physiology and Medicine : Oxygen and Living Processes, An Interdisciplinary Approach.

Editor : Daniel L. Gilbert.

ISBN : 0-387-90554-5, 3-540-90554-5

Springer-Verlag, New York, Heidelberg Berlin.

In last two decades a number of multidisciplinary studies have been carried out on the reactive forms of oxygen: their origin in living systems, interaction with free radicals and enzyme systems and their toxicity in animal and man. However, there has been a lack of comprehensive monographs dealing with such a wide spectrum of knowledge. This lack is fulfilled with the publication of above monograph.

The first part of the monograph covers general aspects of oxygen. D. L. Gilbert has presented detailed perspectives on the history of oxygen and life and significance of oxygen on earth. R. Gerschman has traced the origin and development of the free radical theory of oxygen toxicity. S. Kanfer and N. J. Turo have discussed, in detail, chemistry of active forms of oxygen with an emphasis on interaction among the different research interests. The origin and astronomical history of terrestrial oxygen is very well documented by G. H. Herbig.

The second part of the monograph deals with biology of oxygen. This covers

a wide range of current topics like photosynthetic oxygen production, oxygen toxicity in unicellular organisms, oxygen exchange and biochemistry of oxygen toxicity in metazoa, facilitated oxygen diffusion by carriers, reaction of oxygen with the cytochrome oxidase system and anti-oxidant defences. I. Fridovich has presented an excellent review of progress in our knowledge of superoxide radicals and superoxide dismutases. The discussion on the different forms of superoxide dismutases and their physiological roles is very well elucidated.

The third part deals with human aspects of oxygen. G. L. Huber and D. B. Drath have reviewed the structural and functional effects of high levels of oxygen on the lungs of humans and experimental animals. This review is exhaustive and consists of discussion on the lung as the primary target organ in oxygen toxicity, clinical manifestation of the toxicity, lung function and adaptive changes taking place in the host subjected to the toxic effects of high-oxygen tensions. Many aspects of oxygen toxi-

city and hypoxia occurring in closed environmental systems are covered by K. E. Schaefer. An important aspect of oxygen tension, in clinical situation, is very well discussed by I.A. Silver. The last part of monograph presents an overall biological view on oxygen by D. L. Gilbert.

Thus, this comprehensive monograph covers interdisciplinary aspects of oxygen

in living processes and therefore is an ideal source for those interested in chemistry and function of different forms of oxygen, free radicals and associated biochemical markers sensitive to oxygen status in various experimental and pathological conditions.

A. B. Kulkarni, Ph. D.
Dept. of Biochemistry.

Book Review

Title: Reviews of Physiology, Biochemistry & Pharmacology Vol. 92.

Editors: R. H. Adrian, H. Zur Hausen, E. Helmrich, H. Holzer, R. Jung, O. Kraye, R. J. Linden, P. A. Miescher, J. Piiper, H. Rasmussen U. Trendelenberg, K. U. Urich, W. Vogt, A. Weber.

ISBN — 3-540-11105-0, 0-387-11105-0

Springer Verlag, Berlin, Heidelberg, New York.

The 92 volume of the series of reviews contain three reviews, the first of which concerns toxins acting on Na^+ transport. Excitable cells maintain a concentration gradient across their walls, with respect to Na^+ and K^+ . This gradient is an important factor in the maintenance of the resting membrane potential and the excitability of the cell. While maintenance of the concentration gradient is essential to the continued excitability of the cell, it is itself maintained by Na^+ K^+ pumps. During the passage of action potential, the gradient disappears over the area. After the passage of the spike the gradient is reestablished after a latent period during which the cell is said to be in a refractory state. Development of the action potential and subsequent repolarization entails transfer of Na^+ through the sodium channels.

A wide variety of agents, toxins and natural alkaloids modify the excitability of cells, by interfering with depolarization or repolarization. While these affect the smooth muscle, skeletal muscle and neurons, their effects on the myocardium have attracted maximum attention.

Toxins and other agents which affect Na^+ transport do so either by inhibiting Na^+ transport through the channels or maintaining open state channels prolonging the duration of Na^+ influx. P. Houserjager has confined his review to toxins and agents which prolong the open state of the Na^+ channels. The review contains reports on mode of action of the toxins and their cardiac effects. Since most Na^+ channel gate toxins have a positive inotropic effect on the heart, the toxins have been compared to other cardiotonic agents.

The second review by Knoz and Haas concerns factors influencing renal sodium reabsorption. Increased sodium excretion has been observed, since long, consequential to extracellular vascular expansion though there is no unanimity over the exact mechanisms of increased excretion. The authors have done well to bring together various experimental observations and attempted an objective review of the same. Use of extracellular vascular expansion affects a host of physical, haemodynamic and other factors.

(Continued on page 49)

Book Review

Title : Reviews of Physiology, Biochemistry, and Pharmacology Vol. 91.

Editors : R. H. Adrian, Cambridge, H. Zur Hausen, Freiburg, E. Helmreich, Wurzburg, H. Holzer, Freiburg, R. Tung, Freiberg, O. Kraye, Boston, R. J. Linden, Leeds, P. A. Miescher, Geneve, J. Püper, Gottingen, H. Rasmussen, New Haven, A. E. Renold, Geneve, U. L. Trendelenburg, Würzburg, K. Ullrich, Frankfurt/M. W. Vogt, Gottingen, A. Weber, Philadelphia.

Cloth Binding pages 227

ISB N 3-540-10961-7 Springer-Verlag, Berlin Heidelberg, New York.

ISB N 0-387-10967-7 Springer-Verlag, Heidelberg, Berlin, New York.

This volume of the book from the series of "Reviews of Physiology, Biochemistry and Pharmacology", focuses on three different topics. The very first topic is, by H. Favre and M. S. Briker, on the pathology of marginal renal function. Homeostasis is a natural phenomenon which is observed in this case also. In early stages of chronic renal disorders, almost all kidney function are maintained at normal levels. Although excretions of different solutes like K^+ , Na^+ , PO_4^{3-} are maintained constants, the manners in which they respond to the situations, are different in each case. These different methods of response of these solutes are illustrated here very well along with discussion about acid — base balance. Comprehensive account of the different hormones, these probably affect the reabsorption of these ions, as well as that of the methods used the "glucose titration studies" have been given in quite detail.

The second chapter by G. Hoeman deals with all the aspects of Human Thyroxine Binding Globulin (Human

TBG). This chapter illustrates all biochemical aspects of human TBC like isolation, structure, metabolism, physiological role etc. This glycoprotein seems to be unlucky in the sense that it has no clinical diagnostic application as such and even if it is present in reduced amount or totally absent, it does not interfere in the total metabolism of hormones T_4 & T_3 apparently and therefore has been paid little attention nowadays, but there are some acute phases in its investigations which are quite challenging to the biochemists as is always the case in other investigations.

Phospholipase A_2 enzyme is widely distributed in nature. Extensive discussion of the structure of the enzyme and that of the mechanism of the action of the enzyme has been offered here in the third chapter by H. M. Verheij, A. J. Slotboom, G. H. de Haas. Although being very stable compared to the other enzymes difficulties arise in the purification of the enzyme due to presence of isoenzymes and therefore

making the determination of the actual 3-D structure of it difficult. Even in the kinetic analysis of hydrolysis of aggregated substrate by his enzyme, it requires an additional binding step of the enzyme to the lipid water interface, prior to the Michaelis-Menten Complex formation. This complicates the interpretation of the results of kinetic analysis in terms of well defined rate constant and binding constant. Giving account of all the practical difficulties comparative data of different analyses like kinetic analysis, chemical modification studies, direct binding studies of Ca^{2+} and substrate analogs and X-ray analysis has been presented in detail. Although no details of experimental procedures are given extensive discussion about the facts available, along with extensive bibliography are available.

One thing that strikes the reader's mind is the title of the chapter

which reads "Structure and Function of Phospholipase A_2 ". The chapter includes a discussion about the possible mechanism of the catalytic activity of enzyme at the molecular level, along with comparative studies on the structures of Phospholipase A_2 enzymes from different sources in nature, but it does not mention the functions of the enzyme in the body. Therefore it could have been very aptly named as "Structure and Mechanism of Catalytic activity of Phospholipase A_2 ".

Over all, it can be said that the credit definitely goes to the authors of these different chapters as they have tried to make this volume useful along with precise presentation of work done in respective subjects, for both students and experts, alike in the fields.

— U. N. Katkar

(Continued from page 47)

tors, any or all of which may be responsible for increased Na^+ excretion.

Animal experiments, using the entire systems, or isolated nephrons have been used with success to investigate the relative importance of each factor in the Na^+ excretory process. The results of such experiments cannot be extrapolated with ease, since the animals have undergone significant surgical trauma and have been studied under anaesthesia. These factors make it difficult to clearly assess the contribution of the various factors. The authors themselves concede that the exact mechanism is yet to be clearly under-

stood, yet they manage to evoke sufficient interest in the problem.

The last review is an excellent source for current information on experimental endeavours on the immune system with particular reference to coordination and modification by hormones. The authors have laid stress very rightly so on the thymus with a coverage of the adrenal cortex, the thyroid, the gonads and the adenohypophysis. Since the emergence of the immune system in the control of disease this review is of immense value for students, teachers, and researchers in the field of immunology.

R. B. Ghooi
Hema Tiwari

Title : Membrane proteins — A laboratory manual.

Editors : Azzi A., Brodbeck O. and Zabler P. 76 figures, 256 pages Soft Cover
DM49 Springer Verlag, Berlin Heidelberg, New York. ISBN 3-540-10749-5.

The editors have put together the teaching protocols used at an advanced course on membrane proteins with some chapter on techniques not used there added to complete the score. With this, they hope to present both an introductory manual of biochemical methods in membrane biology, and a readymade 'advanced course' for interested institutions.

Each section represents a major facet of the field. The first, appropriately enough, describes analytical techniques — electrophoretic, immunological and chromatographic — used in studying protein and lipid profiles of membranes — the basic data about any membrane system to be characterised.

The second section deals with a major preoccupation of membrane biochemists — the isolation of biologically active proteins. Examples have been given of a wide range of proteins, from the preparation of spectrin free vesicles from red blood cells (not, strictly speaking, a protein isolation experiment) through complex receptors like histocompatibility antigens to enzymes. The methods depend predominantly on affinity chromatography and gel filtration techniques.

Reconstitution, the subject of the next group of experiments, is increasingly being used to create an approximately natural 'setting' for membrane proteins. Most experiments have used the general approach of solubilisation by a nonionic detergent followed by incorporation into (or on to) phospholipid vesicles. The

subsequent section complements this reconstitution approach by giving some instances of selective labelling of hydrophobic domains in order to study the structural orientation of amphiphilic membrane proteins.

The last section describes some special techniques in protein and protein-lipid characterisation. Spectral techniques like spin labelling and fluorescent spectroscopy have been discussed lucidly. There are some interesting biophysical approaches too. There is a study of supramolecular protein, lipid organisation at phase interphases using surface pressure as the parameter. The final chapter on electron microscopic techniques for liposomes testifies the pivotal role the artificial phospholipid vesicle has played in our understanding of biological membranes.

The theoretical introduction to most experiments is succinct and concise. The details of the experiments have been methodically and painstakingly described, though the lists of chemicals and equipment required put any such 'advanced course' beyond the purse of most institutions in this country. What the volume certainly serves as, however, is an excellent compendium of membrane technology to which the novice membrane biologist in distress can turn in order to see what alternative approaches may come to his aid. And as such a reference source it is an admirable volume.

S. Rath.

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

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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.

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