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Editorial

From the point of view of bacteriologists, the second half of the nineteenth century was probably the most exciting of periods. For, it was in this period that the basis for modern day bacteriology, and also immunology was laid down. It was an age of giants, dominated by men like Pasteur, Koch, Roux, Metchnikoff, Ehrlich and Haffkine.

A hundred years ago, in 1882, a ray of hope dawned, for the millions of patients of tuberculosis, for Dr. Robert Koch had succeeded in isolating the tuberculosis bacilli. For those working in the field of communicable disease, this feat heralded an era which made immunological control of this killer disease, a not-remote possibility. It would be a fitting tribute to the work of Dr. Koch, if today, a hundred years after his discovery, we were to recall his life and draw inspiration, to work for the ailing millions.

Robert Koch was born in 1843 in Klausthal, a peaceful German village, from where he went to Gottingen for his studies in Medicine. In 1866 he graduated as a Doctor of Medicine from the University of Gottingen, and had romantic ideas of roaming around the world as a ship's doctor or a military surgeon. Fate, however had different things in store for him. He joined initially, a not very interesting job of looking after the insane in an asylum at Hamburg. Probably, the best outcome of his stint in Hamburg was his meeting and subsequent marriage with Emmy Froth, who stood by him all his life and supported him in her own quiet way.

As a conciliatory gesture, Koch gave up his ideas of globe trotting and settled down to medical practice in a series of villages cut off from the world of research and serious bacteriology. A significant turn in his life, was a present he received from his wife on his 28th birthday — a microscope. Koch fiddled with it — a toy which relaxed him of rigours of a frustrating practice.

An epidemic of anthrax raged the German farms of the time, and as was the practice of Koch, to scrutinize every material under the microscope, blood of anthrax affected animals, came to be studied. On careful repeated examination Koch observed the presence of a large number of rod shaped particles in blood smears of infected animals, which made him suspect that they were bacilli. Armed with this idea he began the study of smears from healthy animals. Much to his delight he found no

such bacilli. The next step was, naturally, to determine whether the rods were alive. For this, after a series of unsuccessful attempts he managed to infect mice with infected sheep blood.

The work of Koch on anthrax continued, and in its course he developed the hanging drop method to culture the bacilli. With this technique in hand he proceeded to prove that the animals suffering from anthrax had the bacilli in their blood, that their blood could infect other animals, that the bacilli could be cultured and the cultured bacilli could produce the disease. Modern bacteriologists apply the same tests, for every new organisms they find and these tests have come to be known as Koch's postulates to be applied to every microbe, to establish its pathogenicity.

Koch's work would have probably faded away into insignificance, had he rested on the laurels achieved through this feat. But not Koch, he plugged on and on till he could convince the leaders of science, through the good offices of whom he secured the post of Extraordinary Associate to the Imperial Health Office.

Along with this high sounding post came facilities of a well equipped laboratory, grants and assistants. From this laboratory he was able to spread his theory of germs till the world knew of it. It was also in this place that he established the technique of solid cultures and the isolation of microbes in pure cultures.

Koch then turned towards the killer disease which accounted for almost 15% of all deaths in Europe and America. The work on tuberculosis was in no way as easy as that on Anthrax, but with the facilities in his new laboratory and freed from tiresome clinical practice Koch tugged on. Koch began his rounds of hospitals to beg and borrow tissues of persons who had died of consumption. Trying out various strains he focussed his microscope on materials of tubercles, till he found thin bacilli. His initial reaction was 'Ah! they are pretty.'

Koch collected these bacilli in larger numbers and infected guinea pigs and rabbits. For days nothing happened, but after a lag period the animals died with the symptoms of T.B. Koch later managed to isolate, culture and reinfect animals, thus establishing for the second time the cause and effect relationship between a germ and a disease.

On the 24th March 1882, a meeting of the Physiological Society was held in Berlin. Among the delegates were Virchow, and all other heavy-weights of German science. Koch rose to his feet, selected his papers and spoke to them of his researches on tuberculosis. At the end he sat

down to await a discussion on his work but none was forthcoming. All eyes turned towards Virchow, the audience waited. For once the leader of science had nothing to say, Virchow stood up, put on his hat and left the room.

Another task accomplished Koch turned his attention towards cholera. Work on cholera was simultaneously going on in Germany and France, led by two men, Koch and Pasteur, who hated each other sincerely and enthusiastically. In 1883 cholera had entered Europe and was raging in Egypt. Pasteur sent Thullier, his young assistant, with the French Commission to Egypt. Koch and Gaffky of the German Commission were also there to study cholera. On his return Koch wrote to Berlin saying that he had succeeded in isolating the cholera organism. He further requested to be sent to India.

Koch arrived in Bombay in 1897 as the head of the German delegation and received by Dr. W. M. Haffkine. During his stay in India he visited areas where plague was raging. Working with Haffkine he studied the epidemiology of plague, Koch during his stay succeeded in giving Haffkine renewed enthusiasm to continue, providing him with the necessary critical evaluation of his work.

He was awarded the Nobel Prize in Medicine for his work in connection with tuberculosis in 1905. His later work was mainly on plague, sleeping sickness, malaria. He died on May 27, 1910 in Baden-Baden.

Natural Immunity against Tetanus Part II : Possible role of Non Toxigenic *Clostridium tetani* in it

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The possibility of non-toxigenic *Clostridium tetani* inducing natural immunity against tetanus was considered because of isolation of non-toxigenic strains from faeces of non-immunised individuals whose sera provided passive protection against challenge with tetanus toxin in mouse protection test. This led to the isolation of extracellular (b) cytoplasmic (c) and cell wall (d) proteins, of a non-toxigenic isolate of *Cl. tetani*. These proteins were injected subcutaneously in guinea pigs to produce antisera against the respective immunogens. The respective antisera were mixed with 4, 8 and 12 MLD of the toxin and injected in mice for passive mouse protection test. The actively immunized guinea-pigs were challenged with one MLD of the toxin. The results of these experiments showed that antisera against (b) and (c) provided protection in mice, against challenge with tetanus toxin. The guinea-pigs immunized actively with these antigens also showed protection against the toxin. These data suggested that even a non-toxigenic strain of *Cl. tetani* can induce antitoxin immunity.

Introduction

Our previous study (1) on a small group of non-immunized adivasis of Maharashtra State showed that about 48% sera of the population contained protective antibody against tetanus toxin when tested by passive mouse protection test. Stool culture of most of the population yielded growth of toxigenic and non-toxigenic *Cl. tetani*. This suggested that both type of the strains were capable of stimulation of antibody formation. In this study this property of a non-toxigenic isolate was tested.

Materials and Methods

Two strains of *Cl. tetani* one toxigenic and another non-toxigenic isolated from

samples of faeces as reported (1) were employed.

The two isolated were grown in thio-glycollate both at 37°C for 7-10 days; each growth was centrifuged at 5000 gm at 4-5°C for 30 minutes; supernatant (a) and (b) representing respectively the extracellular product of the toxigenic and non-toxigenic strains were collected. The deposit of the latter was mixed well in sterile normal saline; centrifuged at low speed (200-300 r.p.m.) for 15 minutes; the supernatant discarded and the bacterial deposit was disrupted by sonication. This material was centrifuged as before in cold; supernatant (c) and deposit (d) were separated and they represented the cytoplasmic and cell wall fractions of the

organism, respectively (2). The supernatant (c) was sterilized by filtration. The protein contents of (b), (c), (d) and a commercial preparation of tetanus toxoid (Haffkine Bio-Pharmaceutical Corp.) was estimated (3). Polyacrylamide gel electrophoresis was carried (4) for (a), (b) and a preparation of crude tetanus toxin obtained from Haffkine Bio-pharmaceutical Corporation Limited (4).

Preparation of antisera — Antisera were prepared against (b), (c), (d) and the toxoid (table I) by injecting the antigens subcutaneously (s.c.) in guinea-pigs (250-300 g/wt) at interval of one month and for each antigen 6 animals were employed. These animals were bled 15 days after the last dose, sera were separated and stored at 4°C till used for serological test.

Gel diffusion test — This was carried out by method of Ouchterlony for the above 4 antisera against the antigens used for immunization.

Passive mouse protection test — The above 4 antisera were tested for this property but prior to this minimum lethal dose (MLD) of the crude toxin was determined (table II) in mice (Kasauli strain 18-20 g/wt). Then 4, 8 and 12 MLD of toxin were mixed with equal volume of the 4 antisera separately, incubated at room temperature for 45 minutes and 0.5 ml of each mixture injected s.c., in mice. For each MLD 3 mice were used and controls of peptone, toxin and antitoxin maintained.

Active guinea-pig protection test — as before MLD of toxin for guinea pigs (table II) was determined then immunised guinea-pigs were challenged with one MLD of the toxin to test the immunity of the animals.

Results

Polyacrylamide gel electrophoresis — Fig. 1 shows that (b) possessed one lower band like extracellular protein (a) of toxigenic strain and the latter possessed two upper bands like crude tetanus toxin.

Antisera — Two doses of toxoid were required to produce an adequate amount of antibody which gave a single precipitation line with the antiven. On the other hand for the remaining antigens (b, c, d) three doses were required to produce the same results with the respective antiserum.

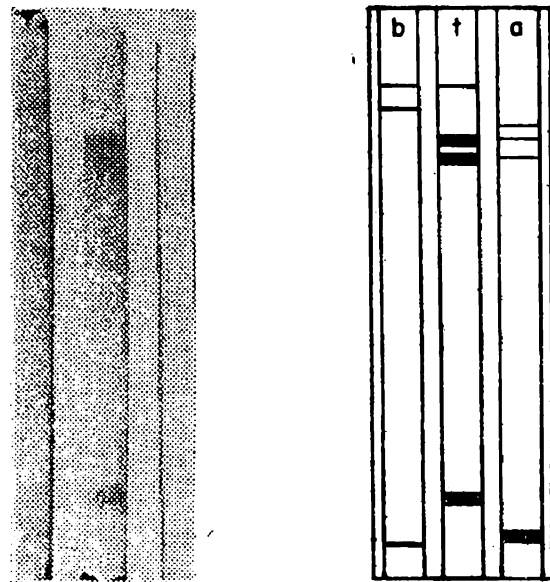


Fig. 1

Table I

Antigen used in antisera preparation

Antigens	Protein concentration mg/ml	Doses by S. C. route
Toxoid	0.3921	Two
(b)	1.258	Three
(c)	0.471	-do-
(d)	0.421	-do-

Table II
MLD of toxin in mice & guinea-pigs

Toxin dils	Vol(m) Inj	Death of animals in							
		24 hrs		48 hrs		72 hrs		96 hrs	
		mice	G. pigs	mice	G. pigs	mice	G. pigs	mice	G. pig.
1X10 ⁻¹	0.5	DDD							
1X10 ⁻²	0.5	DDD							
1X10 ⁻³	0.5			DDD	DD—		—D		
1X10 ⁻⁴	0.5			—D	—	DD	DDD		
1X10 ⁻⁵	0.5			—	—	DDD	D—		—D.
1X10 ⁻⁶	0.5			—	—	—D	—	DD	—

D— indicates death on number of animals ; — no death

Table III
Passive mouse protection test

Materials (A)	Vol of (A)	MLD of toxin (B)	VO (B)	Mixt of (A) & (B) inject	Death in			
					24 hrs	48 hrs	72 hrs	96 hrs
Antisera (b)	1.0 ml	4	1.0 ml	0.5 ml	—	—	—	—
Antisera	1.0 ml	8	1.0 ml	0.5 ml	—	—	—	—
Antisera	1.0 ml	12	1.0 ml	0.5 ml	—	—	—	—
Antisera (c)	1.0 ml	4	1.0 ml	0.5 ml	—	—	—	—
Antisera	1.0 ml	8	1.0 ml	0.5 ml	—	—	—	—
Antisera	1.0 ml	12	1.0 ml	0.5 ml	—	—	DDD	—
Antisera (d)	1.0 ml	4	1.0 ml	0.5 ml	—	—	—D	DD
Antisera	1.0 ml	8	1.0 ml	0.5 ml	—	—	DDD	—
Peptone	1.0 ml	4	1.0 ml	0.5 ml	—	—	—	DDD
Peptone	1.0 ml	8	1.0 ml	0.5 ml	—	—	DDD	—
Toxin	1.0 ml	2	1.0 ml	0.5 ml	DDD	—	—	—
Antitoxin	1.0 ml	4	1.0 ml	0.5 ml	—	—	—	—
Antitoxin	1.0 ml	8	1.0 ml	0.5 ml	—	—	—	—
Antitoxin	1.0 ml	12	1.0 ml	0.5 ml	—	—	—	—

D,— means number of animals dying and living, respectively.

Gel diffusion test — Each antiserum produced a single precipitation line with the respective antigens but antisera (b, c, d) failed to give the same reaction with the tetanus toxoid.

Passive mouse protection test — The MLD of the toxin for mice was 1×10^5 (table II) and antisera (b) and (c) provided protection against 3 and 2 MLD of the toxin, respectively. In control animals, peptone provided protection against 1 and 2 MLD of toxin up to 96 and 72 hours, respectively while 3.0 MLD of the toxin alone killed the animals within 24 hours.

Active guinea-pig protection test — MLD of the toxin for the animals 1×10^{-4} (table II). The animals immunised with toxoid (b) and (c) antigens survived with one MLD of the toxin but after one month the latter two groups of animals died. This indicated that the latter two antigens provided partial protection as compared to the animals immunised with the toxoid and the cellular (d) fraction did not provide any protection against the toxin.

Discussion

It has been observed (5,6,7) that about 30-80% of human population living in highly polluted tetanus area may carry naturally acquired tetanus immunity which might explain as to why these people respond well to first dose of a tetanus toxoid and perhaps do not need artificially immunization (8) against tetanus. Human (9) and animal (5) experiments suggest that the probable mechanism of immunity, is ingestion of tetanus spores and a long-delayed, continuous or intermittent liberation of sub-lethal doses of toxin in the intestinal tract.

The results of this study suggested that even proteins of a non-toxicogenic

strain of *Cl. Tetani* could provide natural immunity against the toxin of the organism. The proteins concerned were located extra and intra-cellularly in the organism and were immunogenic enough to provide protection in animals against toxin of the bacilli. It is interesting to note that *Cl. tetani* toxin contains extra and intracellular components (10), so it was expected that extra cellular (b) and intra common cellular (c) proteins of the non-toxicogenic strain to have some component common with the proteins of the toxin. The presence of a common lower band in electrophoresis of (a) and (b) of extra-cellular products of toxicogenic and non-toxicogenic (fig. 1) suggested the same possibility. Electrophoresis of cytoplasmic fraction (c) was not done but antiserum raised against it provided protection to the animals against challenge of the toxin. The antisera against (b) and (c) failed to give a precipitation line against the crude toxin, possibly the amount of antibody produced in the animals against the respective antigens was not adequate to produce a precipitate on agar but provided adequate protection to the animals against challenge of the toxin. This experiment was limited to one non-toxicogenic strain and was given parenterally, so cannot explain the behaviour of other strains by the former route or orally. Further studies will be carried to study this problem in these aspects.

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Studies on anaphylaxis-II. A selective agonist of the receptor controlling mediator release

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Compound 48/80, on intrapedal administration induces release of mediators of anaphylaxis, which cause the formation of a localized oedema. Studies on this oedema indicate that compound E — [2-(3',4'-dihydroxyphenyl) oxymethyl 8,7, dihydroxy 4-oxo-4H(1) benzo furo (3,2-b) pyrone] inhibited the formation of the oedema, and that this inhibition was susceptible to blockade by propranolol. Compound E was found to inhibit antigen induced oedema in sensitized animals, in a similar fashion. The compound was however found to be without any stimulant effects on the cardiac beta 1, or the tracheal beta 2 receptors. On this basis compound E is suggested to be a selective agonist of the receptors controlling mediator release, and that the antianaphylactic beta receptors are distinct from beta 1 and beta 2 receptors.

Introduction

Since the classification of adrenergic receptors into alpha and beta receptors (Ahlquist, 1948), attempts to identify the various pharmacological receptors involved in physiological functions, have been going on. Thus Lands *et al* (1967), subdivided the beta adrenergic receptors into the beta 1 controlling cardiac contractility and rate and beta 2 receptors mediating smooth muscle relaxation.

Brittain *et al* (1970), after studying a large number of newly synthesized beta stimulants, concluded that classification of beta receptors into the beta 1 and beta 2 types was inadequate. They observed differences in the sensitivities of receptor populations to different agents. The

authors however cautioned, that mere quantitative differences in receptor sensitivities are probably due to heterogeneity of receptors, and should not be used as basis to postulate new subtypes of receptors.

Assem and Schild (1973) first reported that the antianaphylactic beta receptors could not be classified either as beta 1 or beta 2 subtypes, but were probably a new class by themselves. While some studies, notably those of Malta and Raper (1975) supported this contention, Butchers *et al* (1979) suggested that the antianaphylactic receptors are of the beta 2 type.

Bhide *et al* (1978) reported the effects of compound E [2-(3',4'-dihydroxyphenyl) oxymethyl -8,7 dihydroxy 4-oxo-4

H(1) benzo furo (3,2-b) pyrone] on mediator release induced by antigens on sensitized tissues, involving IgE type of antibodies. The workers had failed to elucidate the mechanism of action of the compound, but postulated inhibition of phosphodiesterase.

A simple method for the study of mediator release using compound 48/80 was described by Ghooi et al (1980, 1981). This method was employed to reinvestigate the effects of compound E, with the object of identifying the site of action of compound E. A chance observation on the effects of the compound in propranolol pretreated animals prompted a deeper study into the effects of the compound with respect to its antianaphylactic and other beta adrenergic effects.

Materials and Methods

Compound E was synthesized by the Late Prof. N. V. Subba Rao of the Department of Organic Chemistry, Osmania University, Hyderabad. Compound 48/80 was obtained from Sigma Chemical Co., U.S.A., propranolol hydrochloride and isoprenaline sulphate from I.C.I., U.K., disodium cromoglycate (DSCG) from Fison Pharmaceutical Laboratories U.K., and adrenaline from Themis Pharmaceuticals Bombay India. Ovalbumin was prepared by ammonium sulphate precipitation of egg white, and purified by repeated precipitation in the laboratory. Aluminium hydroxide gel was prepared from the dried gel obtained from Haffkine Bio-Pharmaceuticals Corporation Ltd., Bombay India.

Albino mice (N.I.H., strain of either sex, weighing between 18 to 22 gms.),

guinea pigs (random bred, of either sex weighing between 250 to 350 gms.) were obtained from Haffkine Bio-Pharmaceuticals Corporation Ltd. Male mice of C57BL/6 strain weighing between 20 to 22 gms., were obtained from the Director's Laboratory of the Institute.

Paw thickness measurements were made with a pocket thickness gauge (Mitutoyo Manufacturing Co., Japan). The instrument is calibrated in inches with divisions of 0.001 inch. The paw oedema was expressed in Units, each Unit being equal to an increase in paw thickness by 0.001 inches.

The method for the induction and quantitation of oedema has been described previously (Ghooi et al 1980, 1981). Briefly the method is as follows: The paw thickness of the hind paws was first measured, using the gauge, in normal mice. Next, 0.3 mcg 48/80, dissolved in 0.05 ml., saline was injected intrapedally in the right hind paw, while the left paw received only 0.05 ml., saline. Increase in paw thickness was calculated by repeating the measurements at 10 min. after the injections. The difference in the increased thickness of the right and the left paws reflected the oedema formed in response to 48/80. The dose of 48/80 was the optimal dose, and the time for measurements was based on previous reports on 48/80 induced mouse paw oedema.

On this model the following studies were conducted: a. Effects of compound E on 48/80 induced oedema. Compound E was studied both by the intraperitoneal and local routes of administration at three

dose levels. For local use compound E was dissolved in the solution of 48/80 and administered in the right hind paw, the left paw received only compound E dissolved in saline. The compound (E) for intraperitoneal use was administered 30 min. prior to i.p.d., administration of 48/80 and saline. The effects of compound E were also studied at a single dose level in animals pretreated with propranolol, the beta blocker being administered 30 min., prior to 48/80 and saline.

b Effects of isoprenaline and DSCG on 48/80 oedema. Isoprenaline was studied at one dose level using the local route of administration, by incorporating the appropriate amount of the agent in the solution of 48/80 injected in the right paw, the left paw received saline. DSCG was studied at one dose level, intraperitoneally, administered 30 min., prior to the i.p.d., administration of 48/80. Effects of isoprenaline and DSCG were repeated in animals pretreated with propranolol, administered intraperitoneally (1.0 mg/kg) 30 min. prior to the dose of 48/80.

For studies on the effects of compound E on a model of true anaphylaxis, active paw anaphylaxis in mice as described by Ghooi and Bhide (1981) was used. The method is as follows :

1. Sensitization. Male mice of the strain C57BL/6 weighing between 20 and 22 gms were sensitized with 0.25 mg of ovalbumin adsorbed on 6 mg aluminium hydroxide wet gel suspended in 0.5 ml saline. The mixture was injected in two

divided doses on the back of the mice subcutaneously.

2. Challenge. Sensitized mice were challenged on the 12th day of sensitization, using 10 mcg of ovalbumin injected in the right paw, the left receiving only saline (0.05 ml in each paw). Paw oedema was calculated by measuring the paw thickness before and 15 min., after challenge, using the same method as that for 48/80.

Compound E was studied at a three dose level, the doses being administered intraperitoneally, 30 min., prior to antigen challenge. One dose of compound E was repeated in animals pretreated with 1.0 mg/kg propranolol, 30 min., prior.

In the above mentioned experiments, six animals were used for each set, and the results expressed as a mean with its standard error. The Students 't' test was employed to calculate the significance of the data obtained. The effects of compound E on the beta 1 adrenergic receptor were studied using the isolated atria of guinea pigs mounted in Feigan's solution at 30°C. Adrenaline was used as a standard agonist of the tissue. For effects on beta 2 receptors, the isolated tracheal chain of the guinea pig was used. The preparation was set as per the method of Castillo and DeBeer (1949). The basal tone of the tissue was enhanced by incorporating 0.1 mcg/ml carbachol in the Krebs's solution, and isoprenaline was used as a standard agonist. In both sets of experiments described above six tissues each were used and dose response curves built using the mean and its standard deviation.

Results

Compound E caused a dose dependant inhibition of 48/80 induced oedema by local as well as intraperitoneal route of administration. Table 1 shows the effects of the compound by both routes at different dose levels.

Propranolol (1.0 mg/kg) significantly antagonised the effects of the compound E, when the compound was administered locally or intraperitoneally. At this dose level propranolol per se had no effect on 48/80 oedema, these results are shown in table 2.

Isoprenaline (1.0 mcg, local) and DSCG (1 mg/kg i.p.) both inhibited 48/80 oedema. Propranolol interfered with the effects of isoprenaline but had no influence on DSCG induced inhibition of the oedema (Table 3).

Compound E produced a dose dependant inhibition of antigen induced oedema in sensitized animals, this effect was partially antagonised by propranolol, as shown in table 4.

Adrenaline (5 to 20 ng/ml) induced a dose dependant increase in the amplitude of spontaneously contracting guinea pig atria, while compound E was without effect upto a dose of 800 mcg/ml. The effects of the compounds on the atria are shown in Figure 1.

The isolated trachea of guinea pigs responded with dose dependant relaxation to graded doses of isoprenaline, but failed to respond to compound E upto a dose of 800 mcg/ml. Figure 2 shows the effects of isoprenaline (2.5 to 20 ng/ml) and those of compound E (100 to 800 mcg/ml).

Table 1
Effects on compound E on 48/80 mouse paw oedema

Dose of compound E/ route	Oedema(U)	S.E.	% Inhibition
—	28.8	1.22	—
5 mg/kg i.p.	24.8	1.01*	13.9
10 mg/kg i.p.	18.5	1.38**	35.7
20 mg/kg i.p.	8.5	0.48**	71.5
5.6 mcg local	22.0	0.63**	23.6
11.25 mcg local	16.5	1.80**	42.7
22.5 mcg local	4.1.	0.84**	85.5

Done of 48/80 - 0.3 mcg/paw

*P < 0.05

**P < 0.001

Table 2

Effect of compound E on 48/80 oedema in presence and absence of propranolol

Compound E/ Route	Propranolol(i.p.)	Oedema(U)	S.E.	% Inhibition
—	—	28.8	1.22	—
—	1.0 mg/kg	28.0	1.08*	—
10 mg/kg i.p.,	—	18.5	1.38	35.7
10 mg/kg i.p.,	1.0 mg/kg	24.9	0.96**	13.8
22.5 mcg local	—	4.1	0.84	85.5
22.5 mcg local	1.0 mg/kg	22.3	0.85***	23.0

*P > 0.1 (Not significant) P values calculated for propranolol treated and untreated groups.
 **P < 0.05
 ***P < 0.001 Dose of 48/80 - 0.3 mcg/paw

Table 3

Effects of isoprenaline and disodium cromoglycate on 48/80 oedema in presence and absence of propranolol

Compound/dose/route	Propranolol	Oedema(U)	S.E.	% Inhibition
—	—	23.0	0.81	—
Iso 1.0 mcg local	—	6.8	0.96*	70.43
Iso 1.0 mcg local	1 mg/kg i.p.,	18.4	1.80*	20.00
—	—	20.0	1.80	—
DSCG 1 mg/kg i.p.,	—	7.5	1.04**	65.5
DSCG 1 mg/kg i.p.,	1 mg/kg i.p.,	6.4	1.23**	68.0

*P < 0.001 P values calculated for propranolol treated and untreated groups.
 **P > 0.1

Table 4

Effect of compound E on active paw anaphylaxis in mice

Compound	Propranolol	% Inhibition
E i.p., 30 min., prior	i.p.,	
5.0 mg/kg	—	21.5
10.0 mg/kg	—	43.0
20.0 mg/kg	—	76.0
20.0 mg/kg	1 mg/kg	32.0

EFFECT OF DRUGS ON ISOLATED ATRIA

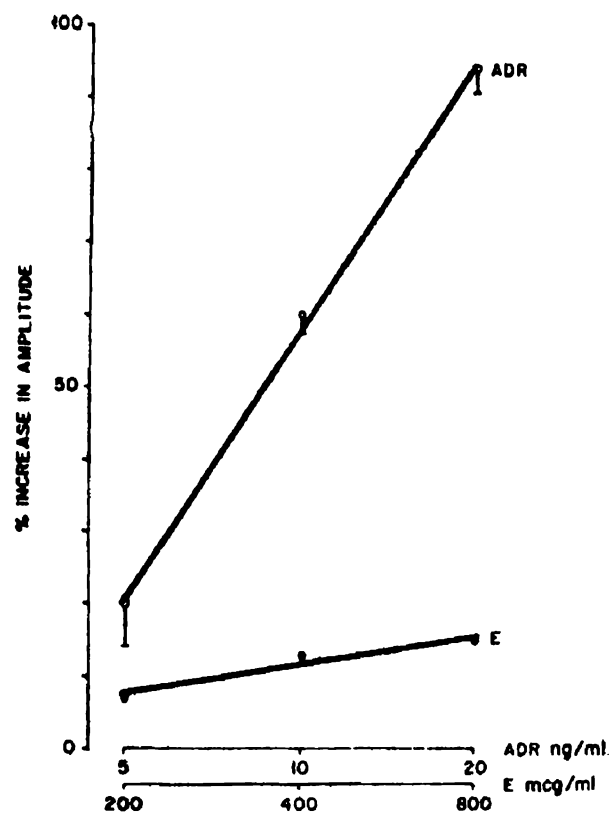


Fig. 1

EFFECT OF DRUGS ON ISOLATED TRACHEAL CHAIN

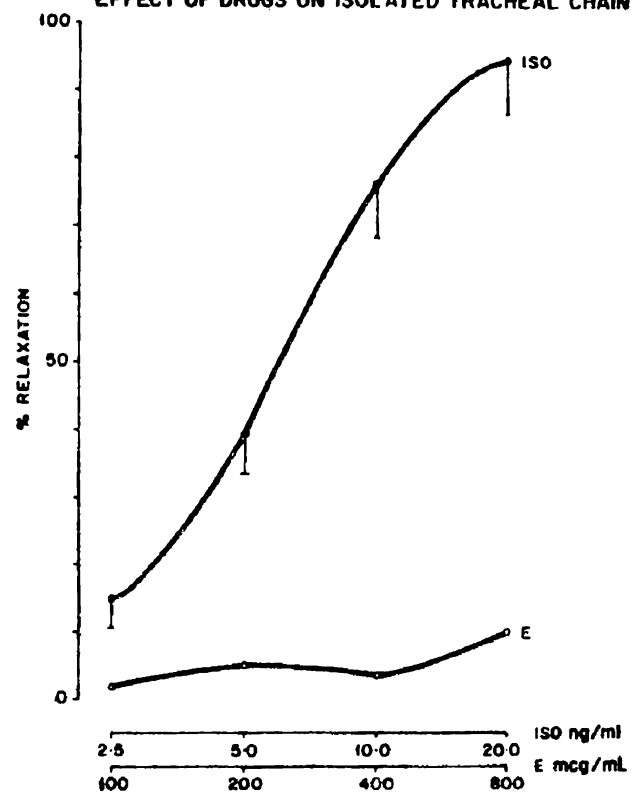


Fig. 2

Discussion

Beta adrenergic receptors are defined as those receptors which are stimulated by isoprenaline and its congeners, and inhibited by propranolol and related agents. In the present studies compound E inhibited 48/80 and antigen induced oedema. Since this effect of compound E is blocked by propranolol, it is justified to suspect that compound E protects against oedema formation by stimulating beta adrenergic receptors. As propranolol at this dose (1.0 mg/kg) inhibited the antianaphylactic effects of isoprenaline but not those of DSCG, the suspicion that compound E acts on beta receptors is confirmed.

Beta adrenergic receptors are widely distributed in the body, and the two known subtypes of beta receptors, viz., beta 1 and beta 2 are concentrated in the cardiac and smooth muscle, respectively, (Lands et al 1967). Catecholamines inhibit antigen induced mediator release, by acting on beta receptors (Assem and Schild 1971), but whether these beta receptors are of the beta 1 or beta 2 subtype is not clear, mainly due to the absence of a specific antianaphylactic beta stimulant.

Studies on compound E suggest that it acts on the antianaphylactic beta receptors, but is virtually without effects on the beta 1 or beta 2 receptors. The data shows a very large difference bet-

ween the sensitivities of different beta receptors for compound E. This we feel cannot be dismissed as another quantitative difference between receptor populations. The disparity revealed is significant enough to be termed as a qualitative difference between antianaphylactic and other beta receptors.

For postulation of a new receptor, the important requirements are the demonstration of a specific agonist and a specific antagonist. Compound E meets the requirement of the specific agonist, studies to fulfil the second requirement are still in progress.

In conclusion, it is suggested that the antianaphylactic beta receptors are distinct from beta 1 or beta 2 receptors. The antianaphylactic receptors have in compound E a specific agonist, and they may be provisionally termed as beta 3 adrenergic receptors.

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Preliminary Communication

Laboratory evaluation of Brodifacoum, a new anticoagulant rodenticide

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The laboratory evaluation of Brodifacoum was carried out against three species of Indian commensal rats, *R. rattus*, *R. norvegicus* and *B. bengalensis*. The compound was found to produce satisfactory mortality when used as a ready to use bait. Two types of tests were carried out. These consisted of 1 day and 3 day feeding no choice tests.

Since the discovery of warfarin (Link, 1945) as an anticoagulant rodenticide, it has proved a major means for an effective rodent control. Other rodenticides were replaced by warfarin because of its chronic antidotal action and want of bait shyness as drawback points. For effective control, multiple bait applications were needed for all these anticoagulant rodenticides and, in general, they were all found to be very effective. (Hayes and Gaines, 1959, Bentley and Larthe, 1959, Greaves and Ayres, 1969).

The discovery of resistance among rodents to warfarin and other anticoagulant compounds resulted in a setback to rodent control operations. *R. norvegicus* in U.K. (Boyle, 1960) as well as house mouse, *Mus musculus* (Rewe and Redfern, 1965, Anon, 1977) and the roof rat, *R. rattus* (Greaves, Rannison and Res-

fern, 1973, Anon 1977) were found to be resistant against warfarin.

To overcome resistance problem, a new rodenticide difenacoum was developed by Redfern, Gill and Halder (1976). This was replaced by Brodifacoum as results found against *R. rattus* and *M. musculus* were less favourable (Halder, Redfern and Rowe, 1975) with 'difenacoum'.

This anticoagulant when tested in the laboratory was found to be effective against warfarin resistant rats, non-resistant rats and mice (Redfern et al. 1976). The investigators claimed that the compound is effective even with single dose.

This anticoagulant was therefore tested against three Indian species of rodents viz. *Rattus-rattus*, *Rattus-norve-*

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gicus and *Bandicota bengalensis* under laboratory conditions.

Materials and Methods

Rodents were obtained through Bombay Municipal Corporation. They were kept in the laboratory for a period of 2 weeks prior to the experiments for acclimatization during which they were fed on the laboratory diet, Rat mouse pellets supplied by Hindustan Lever.

The test animals were kept singly in 'D' shaped cages (30 x 25 x 18 cm). They were starved for 24 hrs. before the start of experimental bait. Animals were provided with expt. bait of 20 gg for 23 hrs. in the first set of experiments. In the second, the experimental bait was offered for 3 days, then rats were maintained on laboratory food for 7 days or until death.

The details regarding sex, weight of the rat, quantity of food consumed and time taken to death were recorded. The bait remaining unconsumed was destroyed and fresh bait offered every day. Water was permitted *ad libitum*.

The rats dying during experiments were dissected to observe the signs of antico-

agulant poisoning such as pale yellow liver, haemorrhage, etc. Only those showing such signs were recorded to have died of rodenticide poisoning.

Three replications of each experiment were made. The experiments undertaken were of the "no choice" type.

Results and discussion

Table I shows results of one day no choice feeding with Brodifacoum bait. It is seen that mortality in P.M. group ranged between 80-100 per cent. Average bait consumption was 13.25 g in *R. rattus*, 15.5 g in *R. norvegicus* and 16.95 g. *B. bengalensis*. Table II presents data on efficacy of Brodifacoum with 3 days no choice feeding test. Mortality was found to be between 90-100 per cent in all species of rats on 7th day.

These studies show that Brodifacoum possesses promising rodenticidal activity against three common species of Indian commensal rodents. Further studies are needed to investigate its acceptability under Indian conditions when used with different bait materials.

Table I

The percentage mortality with Brodifacoum bait (1 day feeding experiment).

Sex and species n = 10	Mean bait intake (gm)	Mean body weight (gm)	% Mortality on days		
			5	7	10
Rr (M)	13.8	98.6	—	50	70
Rr. (F)	12.9	86.9	—	30	80
Rn (M)	14.5	102.6	10	70	80
Rn (F)	16.5	91.3	10	60	80
Bb (M)	17.0	216.9	20	80	100
Bb (F)	16.9	203.7	10	80	100

Total number of rats for each species : 20 per group (10 males and 10 females)

Rr : *Rattus-rattus*Rn : *Rattus-norvegicus*Bb : *Bandicota bengalensis*

n : Number of animals for each species

Table II

The percentage mortality with Brodifacoum bait (3 days feeding experiment).

Sex and species n = 10	Mean bait intake (gm) (3 days)	Mean body weight (gm)	% Mortality on days			
			3	5	7	10
Rr (M)	31.3	109.0	20	70	100	100
Rr (F)	28.0	95.0	—	50	100	100
Rn (M)	40.8	95.4	10	50	90	100
Rn (F)	43.8	89.8	10	50	100	100
Bb (M)	34.2	285.0	10	50	90	100
Bb (F)	28.8	262.0	30	50	90	100

Total number of rats for each species : 20 per group (10 males and 10 females)

Rr : *Rattus-rattus*Rn : *Rattus-norvegicus*Bb : *Bandicota bengalensis*

n : Number of rats for each species

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Investigation of *Lantana camara* Linn (Verbenaceae) Leaves for Larvicidal Activity

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The larvicidal activity of petroleum ether extract of *Lantana camara* Linn leaves (Tantan) was evaluated against IV stage larvae of *Culex p. fatigans*. It was observed that when petroleum ether extract in 0.005 per cent to 0.5 per cent was subjected to mosquito larvicidal activity, very encouraging results were obtained.

Introduction

Lantana camara Linn (Verbenaceae) is very common in many parts of India. It is reported to be a native of tropical America. It has carminative and antiseptic properties. Decoction of this plant is given in tetanus, rheumatism and malaria. It is also taken as a tonic.

The essential oil of *L. camara* has camerene, isocamerene and micranene. Leaves during the flowering and seeding stages yield 0.31 to 0.68% of Lantanine (1). Many authors have done the phytochemical investigation reporting isolation of ketosteroid, triterpenoids, lantic acid, etc. (2-7).

We are reporting preliminary result of *Lantana camara* Linn for mosquito larvicidal activity (*Culex p. fatigans*) and which may finally be developed as a vector control agent.

MATERIALS AND METHODS

Lantana camara Linn leaves (Tantan) collected from Katraj Ghat, Poona District

were dried in shade. The material was then powdered to uniform mesh. One kg powdered material was extracted in percolator with petroleum ether (60-80°C) and the extract was concentrated on controlled temperature water-bath. All solvent was removed and the gummy extract was weighed. The yield was found 2 per cent. Different concentrations were prepared in acetone and mosquito larvicidal activity was evaluated as per method recommended by WHO (8). The IV instar larvae of *Culex fatigans* maintained by the Department of Zoonosis, Haffkine Institute, Bombay were used throughout the experiments. Solvent control and water control were also taken.

RESULTS AND DISCUSSION

Lantana camara Linn petroleum ether extract was evaluated in the concentrations 0.5, 0.25, 0.125, 0.100, 0.050, 0.025, 0.015, 0.010 and 0.005 per cent and mortality count was taken after 24 hours. It can be seen from Table 1 that 0.5% to

0.015% produced 100% mortality whereas, 0.010 and 0.005% showed 72 and 36% mortality respectively.

This preliminary study has yielded a promising indication of *L. camara* being a valuable source of chemicals which may be developed as new vector control agents after further work on its chemical constituents and their activity is accomplished.

Acknowledgement

The authors are thankful to Dr. K. D. Sharma, Director, Haffkine Institute, Bombay for providing necessary facilities for this work and also to Dr. D. M. Renapurkar, Assistant Director, Zoonosis Department for his cooperation. Thanks are also due to Mr. H. V. Nerurkar, Mr. V. R. Patade and Mr. D. T. Jadhav for their sincere help in this work.

Table 1

Mosquito larvicidal activity of petroleum ether extract of *Lantana camara* Linn leaves (Tantan).

Serial No.	% Concentration	% Mortality (24 hrs.)
1.	0.5	100
2.	0.250	100
3.	0.125	100
4.	0.100	100
5.	0.050	100
6.	0.025	100
7.	0.015	100
8.	0.010	72
9.	0.005	36
10.	Solvent control	Nil
11.	Water control	Nil

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BOOK-REVIEW

Title : Reviews of Physiology, Biochemistry and Pharmacology, Vol. 90.

Editors : A. H. Adrian, H. zur Hausen, E. Helmreich, H. Holzer, R. Jung, O. Kraye, R. J. Linden, P. A. Meischer, J. Piiper, H. Rasmussen, A. E. Renold, U. Trendelenburg, K. Ullrich, W. Vogt and A. Weber.

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Springer Verlag, Berlin Heidelberg,
New York.

Von K. Decker, in the opening chapter pays rich tributes to F. Lynen (1911-1979), whose sad demise on August 6, 1979, leaves the world poorer in biochemistry. Feeder Lynen, born in the family of a machine operator, rose up to be, in the words of Konrad Bloch's 'an architect of classical Biochemistry'.

Professor Lynen will go down in the history as a respected scientist, beloved teacher and philosopher. His name shall come to mind when reference is made to Coenzyme A, fatty acid metabolism and energy metabolism. The recipient of many national and international awards, Professor Lynen was awarded the Nobel Prize for Medicine and Physiology in 1964.

At the time of his death Prof. Lynen was the President of the International Union of Biochemistry and held the post of the Director, Max-Planck Institut für Zellchemie. In his 37 year long career,

he guided research and as many as 88 German and other students received his guidance for Diplom and Doctoral work. He had to his credit over 350 research papers.

The first technical chapter of the volume is on the control, modulation and regulation of cell calcium by A. B. Borle. Of the various ions present in the intracellular and extra cellular fluids, calcium has one of the most important roles in the maintenance of the cell integrity and functions, thus the control regulation and modulation are of extreme consequence on the cell. The review opens with the earliest observations of Ringer on the effect of calcium on cardiac contractility, and goes on to trace the historical developments which led to the present understanding of this cation. It deals with, in details total calcium, exchangeable calcium its transport and regulation thereof. The magnitude of calcium transport across the cell and wall is not much different in different cells of different species, though the mechanisms may vary. The review gives vivid descriptions of the variety of techniques available for the study of calcium transport, discussing the drawbacks of each.

Cellular calcium is involved in a vast number of functions which include contraction, secretion and excitability. The cytoplasmic free calcium pool plays many of those roles by its flux across organelle walls. A large number of other factors regulate and modulate the flux of calcium, including sodium, potassium, chloride, cyclic nucleotides and hormones. These form the major part of review, it is un-

fortunate that the author has not focussed on ionophores of calcium.

The mammalian nephron is a major site for reabsorption of calcium, which is quite similar to that of sodium, transport of calcium in the kidney forms the subject of the second review by Augus, Goldfarb and Wasserstein. While a lot of interest has been focussed on the transport of sodium, potassium and chloride in the kidneys, there is a dearth of reviews on the transport systems for calcium. This review fills in the important gap by bringing together the work on the topic in one cover.

The last chapter deals with central receptor chemosensitivity. The H^+ ion concentration is one of the most important factors which affect the respiration in man and animals. Clinically the con-

centration of carbondioxide in the blood is a deciding factor in the maintenance of the blood pH, this concentration being in turn dependent on the respiration. The review discusses in details the areas in the brain associated with the regulation of respiration, their mapping, steriotaxic considerations, and their response to a variety of physiological agents.

Admittedly the review focusses only on pH, since this factor is most important in the maintenance of the respiratory drive, restriction of the discussion is not only excusable, but laudable. The review by Schlafke, is thus a successful attempt to bring together, experimental data available on central receptors concerned with respiration.

Umadevi N. Katkar
R. B. Ghooi.

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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