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CONTENTS

	PAGE
<i>Human Tetanus Immunoglobulin</i> ..	94
J. W. G. SMITH	
<i>Studies on Antagonism between V. cholerae and Sh. flexneri</i> ..	99
F. L. SALDANHA, M. V. SANT, H. I. JHALA AND Y. S. NIMBKAR	
<i>Urinary Tract Infection-I: The Bacterial Spectrum of Urinary Tract Infection in Hospital and Domiciliary Practice and their Drug Resistance Pattern.</i>	103
J. K. DAVE AND D. S. CHITNIS	
<i>Studies on the Effect of Vitamin B₁₂ on Nitrogen Metabolism in Albino Rat</i> ..	108
V. P. SHINDE AND S. D. AMBEGAOKAR	
<i>Transmission Dynamics of Malaria : Quantitative Studies—Part II : Mathematical Nature of Expected Happenings.</i> ..	112
N. R. RAO, O. P. VIG AND S. N. AGARWALA	

Short/Preliminary Communications

<i>Factors affecting the Mouse Protective Value of Casein Hydrolysate Plague Vaccine.</i>	117
M. K. HABBU, H. I. JHALA AND S. S. SOKHEY	
<i>Liver Function Tests in relation to Hepatitis B Antigen in Hepatitis, Leukeamia and Leprosy.</i>	120
JYOTI A. BELLARE, K. V. KULKARNI AND A. J. BAXI	
<i>Laboratory Trials of a New Rodenticide: RH 787</i> ..	123
D. M. RENAPURKAR AND M. V. SANT	

HUMAN TETANUS IMMUNOGLOBULIN

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The replacement of horse tetanus antitoxin by antiserum derived from man is desirable but costly. Its justification depends on the frequency with which hypersensitivity reactions to pepsin-refined horse antitoxin occur, and on the cost. The large-scale production of human antitoxin to completely replace horse antitoxin may not be justifiable at the present time in India since preparation of human antitoxin is costly, and because reactions to horse antitoxin are not common in India when test doses are used to detect hypersensitivity. Moreover, antibiotic prophylaxis may be used to supplement surgical debridement in patients who are sensitive to horse serum. A reasonable compromise is to produce small amounts of human antitoxin for restricted use in patients hypersensitive to horse antitoxin and who require antitoxin either for treatment of tetanus or because they have a severe tetanus-prone wound for which antitoxic prophylaxis may be considered inadvisable.

Tetanus antitoxin prepared from human plasma has replaced horse antitoxin in a number of countries in the past ten years. The human material is more difficult and expensive to procure than animal antitoxin and the justification for its use needs carefully to be considered.

The nature of tetanus antitoxin from human and animal sera

Tetanus antitoxin is an immunoglobulin which appears in the animal serum in response to immunization with a suitable preparation tetanus toxin. Natural, active tetanus toxin is so potent that it is usually impossible to inject even minute amounts without causing fatal tetanus; its lethal dose appears to be much smaller than the amount needed to immunize a man or most animals. For use in immunization the toxin requires to be rendered harmless, but the detoxification must not be too

vigorous or the produce is liable to lose its ability to stimulate formation of antitoxin. A suitable immunizing antigen is made by treating a toxic culture filtrate with formaldehyde to produce formol toxoid. When a horse, man, or other mammal, is injected with tetanus toxoid the antitoxin that appears in the serum is able to neutralise fully potent tetanus toxin. The strength of such antitoxic serum is measured in international units by comparing its neutralising power with that of an internationally agreed standard antiserum, which is, in practice, a horse serum antitoxin. It therefore follows that, in terms of ability to neutralise tetanus toxin, one unit of antitoxin from any species of animal is equivalent to one unit of antitoxin derived from any other animal. Consequently, in neutralising toxin human antitoxin has no advantage over that derived from the horse: an injection of

for example, 10,000 units of horse antitoxin will neutralise just as much toxin as 10,000 units of human antitoxin and, provided they are equally avid, with equal speed and completeness.

Disadvantages of horse antitoxin

Since human antitoxin, as we have seen, has no greater ability to neutralise tetanus toxin than horse antitoxin, the justification for preparing the human material must lie elsewhere in other properties of the antitoxic globulin molecule.

Immunoglobulin molecules not only carry antibody activity but are themselves antigenic; when injected into a different species they may not only be established more quickly than homologous molecules but may also stimulate the formation of antibodies. Thus horse serum antitoxin, not only has a shorter life in man than human antitoxin, but is liable to cause the appearance of anti-horse serum antibodies in the blood. Such antibodies will combine with any horse serum remaining in the patient and the resulting antigen-antibody complexes (horse serum antitoxin—human anti-horse serum) are then rapidly removed from the circulation and eliminated from the body. The patient in whom such immune elimination occurs is left with no protection against tetanus toxin. Moreover, the antigen-antibody complexes are potentially dangerous to the body and may provoke serum sickness. Thus antitoxin prepared in the horse, or in other animals, is not only sometimes of little or no value in man, but is also potentially dangerous—particularly if it has been given before. A patient who has previously had an injection of horse serum may, as a result, produce anti-horse antibodies or may already have them in his circulation; the injected antitoxin is then liable to be eliminated immediately, and the elimination process to be accompanied by

anaphylaxis—which may be fatal. The advantage of antitoxin derived from man lies in its freedom from these drawbacks of the foreign protein.

Although horse antitoxin may itself cause illness and may sometimes be eliminated too rapidly to be of value, it must be stressed that these complications by no means invariably occur, even in individuals who have previously had injections of horse serum. Certainly if crude horse serum is used—i.e. serum drawn from the immunized horse and untreated apart from filtration to ensure its sterility—reactions to its injection in man are not uncommon. To diminish reactivity pepsin-refined horse serum was introduced. The enzyme treatment splits the horse immunoglobulin molecule, separating the major antigenic regions of the molecule from those carrying antitoxic activity—the antitoxic fraction is then purified, often by salt precipitation. The resulting refined antitoxin is appreciably less antigenic than the untreated material and therefore may often be injected without stimulating antibody formation and without causing hypersensitivity reactions.

The frequency with which hypersensitivity reactions follow the injection of pepsin-refined horse antitoxin is not accurately known—it depends to some extent on the criteria used to define reactivity and on the care with which patients are followed-up. The rate of local reactions has been variously reported as between 5 per cent and 50 per cent. (1) Anaphylaxis is rare, perhaps 1 in 10,000 injections, but since it may prove fatal it is one of the main reasons for preferring human antitoxin. The reports on reaction rates referred to are derived from studies in developed countries and there is reason to believe that they may be less frequent in India and other developing countries. Thus in

patients who had not reacted to a test dose an incidence of only 5 per cent was recorded (2). The reason may be that in developed countries horse antitoxin has been widely used, so that many persons have been sensitised by previous injections. It is also possible that proteins may differ in antigenicity in different populations. Whatever the explanation, at the present time reactions to refined horse antitoxin do not appear to present a great problem in most developing countries.

The frequency with which immune elimination of refined horse antitoxin occurs is not known but the little evidence that exists indicates that it might occur in perhaps 10–20 per cent of patients in developed countries. Since immune elimination like hypersensitivity reactions is the result of an immune response to foreign protein, it is also probably less common in developing countries. Consequently, the general replacement of horse antitoxin in order to avoid hypersensitivity reactions and immune elimination may well not be justified in India or other developing countries at the present time. It should however be pointed out that there may be an additional disadvantage for animal antitoxin in treating tetanus patients. There is evidence from experimental work in animals that intrathecal antitoxin is more effective than intravenous antitoxin. In mice, for example antitoxin injected intracerebrally is able to prevent tetanus, in animals previously injected with toxin, at a later stage in the disease process than antitoxin injected intravenously (3). However, horse antitoxin is liable to cause reactions when given intrathecally, whereas human antitoxin appears to be well tolerated by this route. Trials are at present being carried out in Bombay to evaluate intrathecal antitoxin therapy with human antitoxin; should they prove that the method of therapy

has an advantage in man then the provision of the human material for therapy may become important.

Disadvantages of human antitoxin

When the horse is used as a source of tetanus antitoxin a high yield is secured by hyperimmunization of selected animals to provide serum which may contain as much as 1,000 units/ml in the untreated state. Such hyperimmunization is impracticable in man—indeed it may be impossible. Consequently, human serum, even when selected from well-immunized donors, may contain only about 20 units/ml and requires to be concentrated to provide a useful amount of antitoxin in a volume convenient for injection. Even then it is not possible to produce as concentrated a product as horse antitoxin.

Human serum, moreover, may contain viruses pathogenic to man. The only one known to be important in this respect is the virus of serum hepatitis. To ensure that pooled human serum is rendered free from risk of transmitting hepatitis, it requires to be treated. Fortunately the usual process by which the antitoxin is concentrated with cold ethanol gives a product which is safe in this respect. However, the theoretical possibility must exist that other viruses may be contained in human immunoglobulin and it is unwise to regard the material as necessarily completely safe.

From the practical point of view there is an additional drawback; the material at present available cannot safely be administered intravenously since it is liable to cause reactions in a proportion of recipients. These are believed to be due to the presence in the preparation of aggregated molecules of immunoglobulin. Antitoxin is used in clinical medicine in two situations—for prophylaxis of tetanus in injured patients and for treatment of

patients suffering from tetanus. In the latter situation the principal aim of antitoxin therapy is rapidly to neutralise any toxin in the wound, tissues or circulation that has not yet been taken up by the central nervous system. To this end the rapid attainment of a high concentration of circulating antitoxin is desirable—and this is best achieved by intravenous injection. The available evidence suggests that after the intramuscular injection of human antitoxin a peak serum level is not attained until 48 hours or more after injection, and the possibility must exist that in occasional patients the slow absorption of human antitoxin from a subcutaneous or intramuscular injection site may allow transfer into the central nervous system of toxin which might have been neutralised had antitoxin been injected intravenously. Since both human and animal antitoxins neutralise toxin with equal efficiency it is possible that for certain patients—those treated just at the critical time when a lethal increment of toxin is about to be taken up by the CNS—may be treated better with horse antitoxin than human antitoxin. This disadvantage to the therapeutic use of human antitoxin is likely to be overcome fairly soon—perhaps by enzyme treatment similar to that used for horse serum.

In prophylaxis of tetanus the slow absorption of intra-muscularly-injected human antitoxin is unlikely to be a disadvantage. The attainment of a protective level of serum antitoxin is achieved within 48 hours of the administration of the usual dose of 250 units and in almost all patients recently wounded this is probably sufficiently rapid.

Provision of human antitoxin

For prophylaxis of tetanus human antitoxin is undoubtedly much to be preferred to horse antitoxin in that it caused no hypersensitivity reaction and is not liable

to immune elimination. However, its advantage in any community depends on the incidence of hypersensitivity reactions to horse serum antitoxin and on the frequency with which immune elimination of the horse material occurs. The cost of human antitoxin must also be considered. It appears unlikely that hypersensitivity to horse serum is sufficiently common in India at the present time to justify the expense of producing human antitoxin to replace the very large quantities of horse material at present in use for prophylaxis. Moreover, in treatment of tetanus the human immunoglobulin at present available suffers from a drawback that does not clearly weigh its advantages; i.e. cannot be given intravenously.

Human antitoxic immunoglobulin is prepared by the fractionation of plasma; in order to provide a final product in which a prophylactic dose is contained in a reasonably small volume, the plasma to be fractionated should, if possible, contain in excess of 20 units/ml. The concentration of antitoxin to be expected in the serum of immunized persons is on average of the order of 0.1 unit/ml. To secure sera containing 20 units/ml, donors known to be immunized against tetanus need to be boosted with toxoid and bled at the time their antitoxin response is at its height; approximately 2–3 weeks after boosting. Only a proportion of persons will be found to yield serum containing more than 10 units/ml. Consequently, prospective donors require to be screened to select those who respond particularly well.

To produce sufficient human antitoxin to replace the prophylactic doses of horse antitoxin used is clearly a considerable problem and requires plasmapheresis of volunteer donors. Large supplies have been attained with this method by commercial laboratories. However, at the present time the cost of such procedures

may not be justified in many developing countries. On the other hand it is practicable to produce human antitoxin on a small scale, perhaps for treatment of tetanus in patients who are hypersensitive to horse serum, and for prophylaxis in injured patients in whom chemoprophylaxis is not thought to provide adequate protection, e.g. patients with severe wounds. A procedure which has proved satisfactory in a factory, for example, is to ask registered blood donors who have been immunized against tetanus to accept a booster dose of toxoid about three weeks before the blood bank workers are due to call. At the time they give their normal blood donation a small aliquot of serum is separated and

its antitoxin content roughly titrated by a gel-diffusion or haemagglutination-inhibition procedure. Of 250 donors so tasted, 65 (26 per cent) produced an antitoxin response greater than 10 units/ml. The plasma from such blood donations may then be separated and used without fractionation for treatment of tetanus, the therapeutic dose being given by intravenous infusion. Preferably, however, the plasma should be fractionated to give immunoglobulin, since processing by established methods frees the plasma from the risk of transmitting hepatitis. Alternatively the plasma can be screened to exclude sample carrying Australian antigen associated with hepatitis.

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STUDIES ON ANTAGONISM BETWEEN *V. CHOLERAE* AND *SH. FLEXNERI*

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In-vitro and *in-vivo* investigation on antagonism between *V. cholerae* and *Sh. flexneri* under different experimental conditions reveal that the former produces inhibitory agent/s that hinders the growth of the latter and ultimately eliminates it in the mixed culture in liquid medium and in intrainestinally injected infant rabbits. Concurrent presence of *Sh. flexneri* does not deter choleraenicity of *V. cholerae*. Major factors that could play a role in the phenomenon are investigated and the data contributes towards the inference.

Introduction

Reports on the influence exerted by a pathogenic enteric organism on another when concurrently present *in-vitro* and *in-vivo* are extremely rare and no systematic and elaborate study is carried out on the phenomenon.

It is stated(1) that *E. coli* produces an effective antagonist against various bacteria including *Shigella*. Possible inhibitory mechanisms(2) which might enable one bacterial strain or species to prevent the growth of others in the intestine could be: competition for nutrients and oxygen, toxic metabolites and antibiotic substances; changes in pH and lowering oxidation—reduction potential(3) and direct antagonism(4). It is shown that *V. cholerae* eliminated *E. coli*(5) and *S. typhi*(6) *in-vivo* and *in-vitro*.

This presentation projects the observations on investigation on antagonism between *V. cholerae*-Inaba-569 B and *Sh. flexneri*-B-38-10 (Kasauli) *in-vitro* and *in-vivo* under different experimental conditions,

and an allied investigation on the major factors that could come into play.

Materials and Methods

The experimental procedure is similar to as reported earlier (5, 6) except that the MacConkey's agar was incorporated with arabinose replacing lactose on the basis that *Sh. flexneri* has saccharolytic action on arabinose and *V. cholerae* does not ferment it. This facilitated the differential colony count during the *in-vitro* and *in-vivo* study as the colonies of *Sh. flexneri* appeared pinkish and that of *V. cholerae* colourless. Infant rabbit model(7) was used for *in-vivo* studies.

Concisely, the investigation was carried out under different experimental conditions e.g., acidic, neutral and alkaline pH, different levels of bacterial density (10^0 /ml, 10^6 /ml, 10^3 /ml) and in different proportions.

Investigation on major factors was carried out on :

- (i) Production of any inhibitory agent by *V. cholerae* against *Sh. flexneri* in culture-medium or *vice versa*.

- (ii) Nutritional deficiency in the mixed culture-medium that could hinder the growth of *V. cholerae* or *Sh. flexneri*.
- (iii) Growth rate of *V. cholerae* and *Sh. flexneri* individually.
- (iv) Proliferation of *V. cholerae* and *Sh. flexneri* separately at pH 6, 7, 8 and 9.

Results

In-vitro—Table I, II and III presents the observations at high bacterial density (10^9 /ml) at acidic, neutral and alkaline pH respectively. The data reveals that *V. cholerae* eliminated *Sh. flexneri* in about 96 hours. Same trend is observed under other experimental conditions, hence detail data is not furnished in this publication.

TABLE I

Bacterial Interaction : V. cholerae and Sh. flexneri in Nutrient Broth. High Bacterial Density— 10^9 /ml.
ACIDIC pH (6)

Period of incubation at 37°C			1 V. ch.	: 10 Sh. flex.	10 V. ch.	: 1 Sh. flex.	1 V. ch.	: 1 Sh. flex.
24 hours	..	Bact. Count	3.1×10^9	8.0×10^8	3.6×10^9	7.0×10^8	3.2×10^9	6.0×10^8
		pH	6.4		6.4		6.4	
48 hours	..	Bact. count	3.4×10^9	4.0×10^8	3.8×10^9	3.0×10^8	3.4×10^9	3.0×10^8
		pH	6.6		6.6		6.6	
72 hours	..	Bact. count	3.4×10^9	2.0×10^8	3.8×10^9	0.0	3.5×10^9	1.0×10^8
		pH	6.8		6.8		6.8	
96 hours	..	Bact. count	3.4×10^9	0.0	3.9×10^9	0.0	3.6×10^9	0.0
		pH	7.0		7.0		7.0	

TABLE II

Bacterial Interaction : V. cholerae and Sh. flexneri in Nutrient Broth. High Bacterial Density— 10^9 /ml
NEUTRAL pH (7)

Period of incubation at 37°C			1 V. ch.	: 10 Sh. flex.	10 V. ch.	: 1 Sh. flex.	1 V. ch.	: 1 Sh. flex.
24 hours	..	Bact. count	3.5×10^9	7.1×10^8	3.9×10^9	8.0×10^8	3.3×10^9	8.0×10^8
		pH	7.0		7.0		7.0	
48 hours	..	Bact. count	3.7×10^9	6.0×10^8	4.2×10^9	6.0×10^8	3.7×10^9	5.0×10^8
		pH	7.2		7.2		7.2	
72 hours		Bact. count	4.0×10^9	3.0×10^8	4.4×10^9	2.0×10^8	4.0×10^9	1.0×10^8
		pH	7.4		7.4		7.4	
96 hours	..	Bact. count	4.1×10^9	0.0	4.6×10^9	0.0	4.3×10^9	0.0
		pH	7.5		7.5		7.5	

TABLE III

Bacterial Interaction : V. cholerae and Sh. flexneri in Nutrient Broth. High Bacterial Density—10⁹/ml.

ALKALINE pH (8)

Period of incubation at 37°C			1 V. ch.	: 10 Sh. flex.	10 V. ch.	: 1 Sh. flex.	1 V. ch.	: 1 Sh. flex.
24 hours	..	Bact. count	3.6 × 10 ⁹	9.0 × 10 ³	4.1 × 10 ⁹	7.0 × 10 ³	6.0 × 10 ⁹	6.0 × 10 ³
		pH	7.6		7.6		7.6	
48 hours	..	Bact. count	3.9 × 10 ⁹	7.0 × 10 ³	4.4 × 10 ⁹	5.0 × 10 ³	4.0 × 10 ⁹	4.0 × 10 ³
		pH	7.8		7.8		7.8	
72 hours	..	Bact. count	4.0 × 10 ⁹	3.0 × 10 ³	4.5 × 10 ⁹	2.0 × 10 ³	4.1 × 10 ⁹	1.0 × 10 ³
		pH	8.0		8.0		8.0	
96 hours	..	Bact. count	4.1 × 10 ⁹	0.0	4.6 × 10 ⁹	0.0	4.3 × 10 ⁹	0.
		pH	8.2		8.2		8.2	

In-vivo—Typical cholera syndrome was produced in infant rabbits intrainstestinally injected with *V. cholerae* alone or along with *Sh. flexneri* under different experimental conditions. From the intestinal fluid of choleric animals, *V. cholerae* could be isolated abundantly but *Sh. flexneri* could not be detected. Animals injected

with *Sh. flexneri* alone displayed no ill effects and the organism could be isolated from the intestines for about 24 hours. The relevant details are presented in Table IV. Detail observations are not incorporated as the trend of results was typical under other experimental conditions too.

TABLE IV

High Bacterial Density—10⁹/ml.

Bacterial density per ml.			Period in hours death occurred at pH				Cultural isolation from intestinal fluid of	
V. cholerae	Control Proportions	Sh. flexneri	Acidic	Neutral	Alkaline	V. cholerae	Sh. flexneri	
1. 10 ⁹	C +.	0	18	18	16	+	Nil	
2. 10 ⁹	C +	0	18	18	16	+	Nil	
3. 0	C ?	10 ⁹	live	live	live	Nil	+	
4. 0	(N.S.)	0	live	live	live	Nil	Nil	
5. 10 ⁹	10 : 1	10 ⁸	18	18	18	+	Nil	
6. 10 ⁹	10 : 1	10 ⁸	18	18	16	+	Nil	
7. 10 ⁸	1 : 10	10 ⁸	18	18	18	+	Nil	
8. 10 ⁸	1 : 10	10 ⁸	18	18	18	+	Nil	
9. 10 ⁸	1 : 1	10 ⁸	18	16	16	+	Nil	
10. 10 ⁸	1 : 1	10 ⁸	18	16	16	+	Nil	

C + = Positive control. C ? = 'Test' control. N.S. = Blank (Normal Saline).

+ = Isolation of *V. cholerae* or *Sh. flexneri*. Nil = Not isolated.

The allied investigation unravelled:

- (i) *Sh. flexneri* does not grow in the *V. cholerae*-culture-filtrate (Seitz).
- (ii) Addition of fresh medium to the mixed culture medium does not enhance the growth of *Sh. flexneri*.
- (iii) The rate of growth of *Sh. flexneri* is slower than that of *V. cholerae*.
- (iv) Individually, *V. cholerae* and *Sh. flexneri* grow at pH 6, 7, 8, and 9 but the growth of the latter is comparatively less.

Discussion

The data unravels that *V. cholerae* produces inhibitory agent/s in the liquid medium and in the intestines of the infant rabbits that is primarily responsible for the elimination of *Sh. flexneri*.

Observation depicted in Table I, II and III show that in the mixed culture the growth of *Sh. flexneri* is inhibited right from beginning and dwindles down speedily and gets eliminated in about 96 hours under all permutation and combination of experimental conditions and *V. cholerae* gives luxuriant growth. It is indicated that an inhibitory agent is produced by *V. cholerae* against *Sh. flexneri*.

The infant rabbits injected with the mixed culture of *V. cholerae* and *Sh. flexneri* under different experimental conditions, produced a typical cholera syndrome. *V. cholerae* could be isolated practically as a pure culture from the intestinal fluid but *Sh. flexneri* could not be detected; but when *Sh. flexneri* was injected alone, it survived in the intestines of infant rabbits for about 24 hours and the animals showed no signs of illness. It is apparent that an inhibitory factor is produced by *V. cholerae* *in-vivo* too, that is detrimental to the growth of *Sh. flexneri*. Concurrent presence of *Sh. flexneri* does not deter the choleragenicity of *V. cholerae*.

The investigation on the major factors contributed towards the inference on the production of antagonistic agent by *V. cholerae* against *Sh. flexneri* as the latter did not grow in the *V. cholerae*-culture-filtrate (Seitz) and this inhibition could not be attributed to the nutritional deficiency of the medium in which both the organisms were grown together as replenishment with fresh medium did not enhance the growth of *Sh. flexneri*. The rate of growth of *Sh. flexneri* is comparatively slower than that of *V. cholerae*. This could help in the process of elimination. Different levels of pH did not seem to have reckonable role in the phenomenon.

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URINARY TRACT INFECTION-I

The Bacterial Spectrum of Urinary Tract Infection in Hospital and Domiciliary Practice and their Drug Resistance Pattern

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Out of 433 samples of urine analysed from Hospital and private practice, 199 (45.96 per cent) exhibited significant bacteriuria. The isolates were subjected to susceptibility test against seven commonly used antimicrobial drugs viz., Sulfadimidine, Streptomycin, Ampicillin, Chloramphenicol, Tetracycline, Kanamycin and Furadantin. An alarmingly high incidence of multiple drug resistance was observed against Sulfadimidine, Streptomycin, Ampicillin, Chloramphenicol and Tetracycline. There was no significant difference in the drug sensitivity patterns in the strains isolated from Hospital and Domiciliary practice.

Introduction

There has been a steady increase in the incidence of coliform infections since the antimicrobial agents have come into wide use. The changing prevalence of these pathogenic bacteria is most conspicuous in urinary tract infections in which certain species of this group are gaining predominance in recent years. The more disturbing feature is the multiple drug resistance currently observed in these organisms which has created a serious problem in the treatment of urinary tract infections.

Materials and Methods

Four hundred and thirty-three samples were received from an unselected series of patients referred from hospitals and from private practitioners. Midstream urine samples collected by clean catch method in sterile containers were used.

A 3 mm loopful quantity of urine sample was inoculated on MacConkey plate. The total viable bacterial count in the urine sample was determined by the semi-quantitative method of Mohankumar(1). Urine samples showing 10^5 or more viable bacteria per ml of urine were considered to indicate significant bacteriuria(2).

The antibiotic sensitivity tests were carried out by the disc diffusion technique as described by Bauer and Kirby(3). Mueller-Hinton medium was used and the inoculum dose and concentration of the drug per disc strictly followed as per Bauer's method. *E. Coli*, N. C. T. C. 10418, was used as a control to check the accuracy of the test. The isolates were tested against seven antimicrobial agents routinely used against enteric organisms in this laboratory viz., Sulfadimidine, Streptomycin,

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Tetracycline, Chloramphenicol, Ampicillin, Furadantin and Kanamycin.

Results

One hundred and ninety-nine out of 433 samples of urine i.e., 45.9 per cent showed significant bacteriuria. Fifteen out of these 199 samples showed mixed infection with two organisms and two samples with three organisms (Table I). Table II shows the various organisms encountered in the present study. *E. Coli* showed a significant predominance (39 per cent). *Klebsiella* were encountered in 20.2 per cent of cases, *Proteus* in 14.2 per cent of cases and *Pseudomonas* in 8.7 per cent of cases. There was no significant difference in the frequency distribution of pathogens between the hospital and domiciliary practice. Results of antibiotic sensitivity test are

presented in Table III. Figures in parenthesis represent the number of strains tested. It is seen that majority of strains of *E. Coli* were resistant to Sulfadimidine, Streptomycin, Ampicillin, Chloramphenicol and Tetracycline and were sensitive to Furadantin and Kanamycin. Similar pattern was observed for *Klebsiella*. Majority of *Proteus* species were resistant to the above drugs and in addition their resistance to Furadantin and Kanamycin was of higher degree than of *E. Coli* and *Klebsiella*. All the strains of *Pseudomonas* were resistant to the routine drugs tested. Some of the multiple drug resistant strains were tested against Gentamycin. All the *Pseudomonas* strains and others except one strain each of *Proteus* and *Str. faecalis* were found to be sensitive to Gentamycin (Table IV).

TABLE I

Total samples tested	No. of samples without significant bacteriuria	No. of samples with significant bacteriuria	No. of mixed infections with 2 organisms	No. of mixed infections with 3 organisms
433	234 (54%)	199 (45.96%)	15 (3.42%)	2 (0.46%)

TABLE II

Bacterial Spectrum in Urinary Tract Infection

Organisms	Domicile	Hospital	Total	Percentage
<i>E. coli</i>	43 (41.0%)	42 (37.2%)	85	39
<i>Klebsiella</i> sp.	18 (17.1%)	26 (22.5%)	44	20.2
<i>Proteus</i> sp.	18 (17.1%)	21 (18.6%)	39	14.2
<i>Pseudomonas</i> sp.	10 (9.5%)	9 (7.9%)	19	8.7
<i>Str. faecalis</i>	4 (3.8%)	6 (5.3%)	10	4.6
<i>Alk. faecalis</i>	6 (5.7%)	6 (5.3%)	12	5.5
<i>Citrobacter</i> sp.	0 (0.0%)	2 (1.8%)	2	0.9
<i>S. albus</i>	4 (3.8%)	0 (0.0%)	4	1.8
<i>S. aureus</i>	2 (1.9%)	0 (0.0%)	2	0.9
<i>B. anitratum</i>	0 (0.0%)	1 (0.9%)	1	0.5
Total isolates	105	113	218	

Summary

Bacteriological study of 199 cases of Urinary Tract Infection with significant bacteriuria revealed preponderance of Gram negative bacilli. Antibiotic susceptibility tests of isolated strains against seven antimicrobial agents in common use exhibited an alarmingly high degree of Multiple Drug Resistance. There was no significant difference in bacterial spectrum as well as the drug resistance pattern in

the material referred from Hospital and Domiciliary practice.

A brief mention has been made to similar studies on Urinary Tract Infections in India.

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STUDIES ON THE EFFECT OF VITAMIN B₁₂ ON NITROGEN METABOLISM IN ALBINO RAT

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Male albino rats were made deficient in vitamin B₁₂. The deficient animals were given a single dose of vitamin B₁₂ (5 µg/rat) by intraperitoneal injection. Food intake was recorded. Urine and faeces samples were also collected during 72 hours after administration of the vitamin. The animals were then sacrificed. The nitrogenous constituents of urine, liver and serum were determined. Vitamin B₁₂ produces a significant effect on per cent retention of dietary nitrogen and reduces the concentration of urinary urea, creatinine and ammonia nitrogen. This vitamin also lowers significantly the levels of free amino acid nitrogen of liver and serum. The results indicate that vitamin B₁₂ seems to be involved in over all nitrogen metabolism.

It was reported in the previous communication(1) that vitamin B₁₂ promotes the synthesis of liver and serum proteins, haemoglobin, erythrocytes and leucocytes in albino rats fed on 18 per cent casein diet. However, the effect of this vitamin on nitrogen metabolism is not yet systematically elucidated.

It has been shown that the growth rate of variety of animals, such as rats(2), mice(3), hogs(4) and chicks(5) was increased when vitamin B₁₂ was added in the diet. However, the accumulated data suggest that the enhanced growth rate of the animals was the reflection of increased food intake. Chow and Barrows(6) and Black and Bratzler(7) had shown the increased growth rate of the animals receiving vitamin B₁₂ was due to increased food intake, whereas the nitrogen utilization was not altered by giving this vitamin.

On the other hand, several workers(8, 9) had indicated that vitamin B₁₂ improves the utilization of dietary proteins from

several sources such as casein, groundnut protein, egg albumin and cottonseed oil meal in rats. The blood non-protein nitrogen level was also found to be higher in chicks(10) and in rats(11) fed on vitamin B₁₂ deficient diet than in the controls which received the vitamin. They also further reported that vitamin B₁₂ was effective in reducing the blood level of certain amino acids.

Thus, it will be seen from the above reports that the effect of vitamin B₁₂ on growth and nitrogen metabolism is not fully understood and the available literature is also very conflicting. In view of this, the present studies were undertaken to study the role of this vitamin on nitrogen metabolism. This paper presents the data on retention of dietary nitrogen and nitrogen balance, distribution of urinary nitrogenous constituents, and free alpha-amino nitrogen in liver and serum of deficient rats before and after intraperitoneal administration of vitamin B₁₂.

Materials and Methods

Male albino rats of Haffkine strain, four-five weeks old and weighing 35-40 g were made deficient in the vitamin by maintaining them on the basal diet devoid of vitamin B₁₂ for ten weeks as described by Shinde and Ambegaokar (1). These deficient animals were evenly distributed in the two groups. To the deficient group, no vitamin was administered and to the experimental group, a single dose of vitamin B₁₂ (5 µg/rat) was given by intraperitoneal injection.

The rats in both the groups were then transferred immediately into the individual metabolic cages. They were continued to be fed *ad libitum* on basal diet. The food intake was recorded during the 72 hours after administration of the vitamin. The collection of urine and faeces was also made during the same period. Urine was collected in a bottle containing a few millilitres of acetic acid and toluene. It was then diluted to 500 ml with washings in distilled water, filtered and stored in the refrigerator until used for analysis. Faeces were collected every day, pooled and moistened with a few drops of 10 per cent oxalic acid and dried in the hot air oven at 105° C. The dried faeces were weighed, powdered and kept in the desiccator until further use. At the end of 72 hours, when the collection of urine and faeces samples were over, animals from both the groups were sacrificed. Blood was obtained from heart and allowed to clot to obtain serum. The livers were quickly excised and placed in the ice-cold normal saline. They were weighed quickly and made into 20 per cent homogenates with distilled water.

Total nitrogen of urine and faeces was determined by the micro-Kjeldahl procedure. The urine samples were further analysed for the four major nitrogenous constituents viz., urea, creatinine, ammonia

and free alpha-amino acid nitrogen. Ammonia nitrogen was determined by the Nesslerization method of King (12). Creatinine nitrogen was determined by the alkaline picrate method of Foli (13). Urea nitrogen was determined by the colour reaction with the Ehrlich's reagent as described by Yatzidis *et al.* (14). Free alpha-amino acid nitrogen content of urine, serum and liver was estimated by the colour reaction with ninhydrin as the method of Matthews *et al.* (15).

Results

The results of the gain in weight, nitrogen intake, nitrogen balance (nitrogen intake—urinary plus faecal nitrogen) and per cent retention of nitrogen (nitrogen balance $\times$ 100/nitrogen intake) in the body during 72 hours are presented in Table I. The results of partition of urinary nitrogen and free amino acid nitrogen content of liver and serum in the deficient and experimental groups of rats are shown in Tables II and III respectively.

Discussion

It will be seen from the Table I that the nitrogen balance and per cent retention of nitrogen is increased significantly during 72 hours when vitamin B₁₂ is given to the deficient animals. The results in Table II indicate that the reduction in urinary excretion of nitrogen when vitamin B₁₂ was administered in deficient animals is mainly due to reduction in the excretion of urea nitrogen. The reduction in creatinine and ammonia nitrogen is also found significant. These results indicate that the anabolic process in the rat is elevated due to intraperitoneal administration of vitamin B₁₂. The results presented in Table III also show that this vitamin produces a marked reduction in free alpha-amino acid nitrogen of liver and serum. However, there is no effect on free amino acid nitrogen of the urine.

TABLE I

Effect of vitamin B₁₂ on nitrogen balance and per cent retention of dietary nitrogen in albino rats
(Average of four values with standard error)

Administration of vitamin B ₁₂	Gain in weight (g)	Food nitrogen intake (mg)	Urine-N + Faecal-N (mg)	Nitrogen balance (mg/72 hrs)	Percent retention of nitrogen
None (Deficient) ..	10	604 ±23.0	384 ±9.1	220 ±16.0	36.4 ±1.5
Single dose, 5 µg/rat (Experimental) ..	12	621 ±4.8	322 ±11.4	299 ±9.4	48.1 ±1.7
Statistical analysis, values of 't' ..	..	0.72	4.25	4.29	5.16

Values of 't' equal to and greater than 3.17 are significant at 1 per cent level.

TABLE II

Effect of vitamin B₁₂ on urinary nitrogenous constituents of albino rats
(Average of four values with standard error)

Administration of vitamin B ₁₂	Urinary nitrogen excreted during 72 hours (mg/rat)				
	Total	Urea	Creatinine	Ammonia	Free amino acid
None (Deficient) ..	242 ±6.22	165 ±4.82	9.85 ±0.13	23.75 ±0.31	4.40 ±0.26
Single dose, 5 mg/rat (Experimental) ..	211 ±6.77	126 ±1.59	9.05 ±0.17	20.75 ±0.31	4.56 ±0.08
Statistical analysis, values of 't' ..	3.77	7.71	3.77	6.58	0.59

Values of 't' equal to and greater than 3.17 are significant at 1 per cent level.

TABLE III

Free alpha-amino acid nitrogen content of liver and serum of rats after administration of vitamin B₁₂
(Average of four values with standard error)

Administration of vitamin B ₁₂	Final body weight (g)	Weight of liver per 100 g. body weight (g)	Free amino acid nitrogen	
			Liver (mg/g)	Serum (mg/100 ml)
None (Deficient) ..	100	3.85 ±0.12	22.95 ±0.50	10.50 ±0.15
Single dose, 5 µg/rat (Experimental) ..	99	4.03 ±0.09	12.25 ±0.80	9.80 ±0.12
Statistical analysis, values of 't' ..	..	1.65	34.02	3.68

Values of 't' equal to and greater than 3.17 are significant at 1 per cent level.

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TRANSMISSION DYNAMICS OF MALARIA

Quantitative Studies—Part II *Mathematical Nature of Expected Happenings*

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A stochastic model giving the infection rate, the recovery rate and an expression for the probability of natural recovery was presented in Part I. On the basis of this model, the course of events as expected happenings were analysed and presented in this paper.

Expression for the endemic level and the condition for the occurrence of recoveries were obtained as a result of the interaction of the infection rate and the recovery rate. Expressions were derived for the critical degrees of transmission and for the measurement of malaria stability, equilibrium and sensitivity of malaria.

The present study revealed that the index of stability is given by the expression for the probability of an anopheline vector having an infective feeding on man during its life time.

Introduction

In the history of speculation on the causes of explosive outbreaks of disease, William Farr 1840(1) and the nineteenth century mark a period of transition from philosophical to mathematical models of the behaviour and course of epidemics. However, the development of mathematical models with the understanding of specific biological causes to provide a mechanistic explanation of the observations were not pursued until the early twentieth century (2), (3).

In Part I(4), we have presented a stochastic model giving the infection rate, the recovery rate and the resultant probability of natural recovery. In this part, we examine the course of events giving the

level of endemicity and by its operation, the critical degrees of transmission. We also examine the sensitivity of the model and give an expression for the index of stability of malaria transmission.

Materials and Methods

In the development of a stochastic model (4), the variables relating to man, the disease and man's reaction to it and the mode of transmission were described.

Notations used in this publication are same as in Part I(4).

The starting point of this study is the system of differential equations developed by us(4), for the change in the proportion of people affected when stability is reached.

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Using the methods of calculus, expressions are obtained to describe the expected happenings.

Results

The process of increase in malaria is a chain of events. The relationship between inoculation frequency, recovery rate, mortality and the resultant infection rate in a community when the possibility of superinfection was admitted, were presented in Part I(4). The sensitivity of the endemic level to alterations in it will be presented in this Part II.

1. *The endemic level and the condition for the occurrence of recoveries.*—The endemic level is defined as the proportion of population affected when stability is reached and before the common acquisition of material resistance(5). Stability can be reached at the maxima or

minima of U in $\frac{dU}{dt} = 0$. Therefore the

endemic level is the maximum value or the limiting value of the change in the proportion affected. As there are two forms of expressions for the proportion of population affected according as the case when there are recoveries and the case when there are no recoveries(4), so also there will be two forms of expressions for the endemic level viz., the endemic level for (i) the case when there are recoveries and (ii) the case when there are no recoveries. We derive the expressions for these two forms and obtain the condition for the occurrence of recoveries.

(i) *The endemic level for the case when there are recoveries.*—We have obtained an expression for the change in the proportion of population affected, in Part I(4), as:

$$\frac{dU}{dt} = h - rU. \quad \dots (1)$$

We have derived an expression for h the inoculation rate, in Part I(4), as:

$$h = \frac{m p^n a^2 x^2 w^n U}{a x U + v + M}. \quad \dots (2)$$

Substituting for h from (2) in (1), and

equating $\frac{dU}{dt} = 0$, we get the maximum

value of U as:

$$U = \frac{m p^n a^2 x^2 w^n - r(v + M)}{r a x}. \quad (3)$$

or

$$U = L_u = \frac{m p^n w^n a x}{r} - \frac{(v + M)}{a x}. \quad (4)$$

This is the expression for the limiting value of U denoted by L_u called the endemic level when stability is reached and for the case when there are recoveries.

(ii) *The endemic level for the case when there are no recoveries.*—In the case when there are no recoveries, the change in the proportion of population affected is given by the expression [see Part I(4)] as:

$$\frac{dU}{dt} = h(1-U). \quad \dots (5)$$

Substituting for h from (2) in (5), we get

$$\frac{dU}{dt} = \frac{m a^2 x^2 p^n w^n U}{a x U + v + M} (1-U).$$

For the maximum value, $\frac{dU}{dt} = 0$,

which gives:

$$U = L_u = 1.0. \quad \dots (6)$$

The value of U denoted by L_u in (6) gives the endemic level when stability is reached in the case when there are no recoveries. The expressions in (4) and (6) show the levels at which the disease will tend to stabilise itself.

(iii) *The condition for the occurrence of recoveries.*—When the stability is reached,

the change in the proportion affected is given by (4) as:

$$L_u = \frac{m a^2 x^2 p^n w^n - (v + M) r}{r a x} \dots (4)$$

As L_u is the asymptote of U , the equation (4) clearly holds only if the asymptote L_u is less than or equal to 1 (Armitage, 1953 (6)). This condition gives:

$$m a^2 x^2 p^n w^n - (v + M) r \leq r a x$$

or

$$r > \frac{m a^2 x^2 p^n w^n}{a x + v + M}, \text{ i.e., } r > h, \dots (7)$$

when $U = 1$ in the equation (2), that is, if r is greater than or equal to the highest value that can possibly occur. This supports our categorisation that when h is less than r , there are recoveries and when h is greater than or equal to r , there are no recoveries.

2. *The critical degrees of transmission.*—The critical degrees of transmission are the values of the factors responsible for transmission such that the critical values separate those values which would result in maintaining transmission from those which would lead to extinction of the disease or which would result in the progressive reduction of malaria to an utterly negligible level, it being assumed that the probability of a mosquito surviving through each successive day is constant and known(5). The critical levels are given by the explicit values of the respective factors for the zero limit of the first differential of equation (4). Numerically

they are given by the minima of $\frac{dU}{dt} = 0$.

Equating (4) to zero gives:

$$L_u = \frac{m a x p^n w^n}{r} - \frac{(v + M)}{a x} = 0$$

or

$$\frac{m a x p^n w^n}{r} = \frac{(v + M)}{a x} \dots (8)$$

Critical levels can be obtained from equation (8) separately for various factors, such as mosquito density in relation to man 'm'; frequency of bites per night (day) 'ma'; or frequency of infective bites 'ax' which are as under:

(i) Critical level of mosquitoes in relation to man is given by:

$$m = \frac{r(v + M)}{a^2 x^2 p^n w^n} \dots (9)$$

(ii) Critical frequency of bites per night 'ma' is given by:

$$ma = \frac{r(v + M)}{a x^2 p^n w^n} \dots (10)$$

(iii) Critical frequency of infective bites 'ax' is given by:

$$a x = \sqrt{\frac{r(v + M)}{m p^n w^n}} \dots (11)$$

Critical levels for any other factor can be obtained in a similar manner.

3. *The measurement of stability of malaria*—Stability has been defined as a tendency (or rather its absence) towards changes in endemicity level upon environmental changes. The stability of malaria is numerically expressed by the value of the minimum increase in the reproduction rate necessary to raise the limiting value L_u from 0 to 1.0(5). This may be obtained by expressing L_u in terms of Z_0 the basic reproduction rate.

The endemic level, that is, the level of the proportion affected when stability is reached, is shown to be given by the equation (4) as:

$$L_u = \frac{m a x p^n w^n}{r} - \frac{(v + M)}{a x} \dots (4)$$

and the basic reproduction rate Z_0 is given by the equation, fully derived in Part I(4), as:

$$Z_0 = \frac{m a^2 x^2 p^n w^n}{r(v+M)} \quad \dots (12)$$

Rearranging L_u in terms of Z_0 , we get:

$$L_u = \frac{(v+M)}{a x} (Z_0 - 1) \quad \dots (13)$$

If the values of 1 and 0 are successively attributed to L_u , the successive values of Z_0 are:

$$\text{for } L_u=1, Z_0=1 + \frac{a x}{(v+M)}, \quad \dots (14)$$

$$\text{for } L_u=0, Z_0=1.0. \quad \dots (15)$$

Thus the index of stability 'IS' as given by the minimum increase in the basic reproduction rate necessary to raise L_u from Zero to 1.0 is given by:

$$IS = \frac{a x}{(v+M)} \quad \dots (16)$$

This is the measure for the stability of malaria, called stability index. This in fact directly expresses the probability of an anopheline vector having an infective feeding on man during its life time.

4. Equilibrium and sensitivity of malaria.—Equilibrium refers to the level at which the index of the proportion of people affected will stabilise itself at the end of a period of change(5). Equilibrium therefore may be established at zero—that is, by extinction of the disease; at some level between 0 and 1 (0 and 100 per cent) or 1. The expressions of the infection rate for the limiting value and for any time before stability is reached will be expressions for the establishment of equilibrium at different levels.

The establishment of equilibrium between 0 and 1 is given by the equation (4) as:

$$L_u = \frac{m a x p^n w^n}{r} - \frac{(v+M)}{a x} \quad \dots (4)$$

The expressions for the establishment of equilibrium at 0 and 1 are the expressions for the critical levels.

Sensitivity refers to the degree to which the endemic level is altered by a change in one of the controlling factors(5). This is analysed mathematically by the production of partial derivatives of equation (4) for the endemic level with respect to each of the factors in question. For example, (i) the sensitivity level with respect to the mosquito density in relation to man is given by:

$$\frac{dL}{dm} = \frac{L_u + \frac{(v+M)}{a x}}{m} \quad \dots (17)$$

(ii) The sensitivity level in relation to changes in the recovery rate is given by:

$$\frac{dL}{dr} = -\frac{1}{r} \left(L + \frac{(v+M)}{a x} \right) \quad \dots (18)$$

It will be thus seen that all the partial derivatives have the subsidiary expression $(L + [(v+M)/a x])$. We define the index of sensitivity 'SI' as the minimum change in the endemic level which is given by:

$$SI = \frac{(v+M)}{a x} \quad \dots (19)$$

This index relates the sensitivity to the pre-existing equilibrium and to the average number of infective feeds on man which a mosquito takes in the course of its life time.

Discussion

We reviewed Macdonald's theory in Part I and derived an expression for the infection rate in the mosquito taking into consideration the situation of the growing and declining mosquito population. It was also assumed that the infection rate in the

mosquito was dependent on the proportion affected in humans at some time previously. With the revised expression or the infection rate in the mosquito, the expression for the endemic level was obtained and the expected happenings were analysed.

The endemic level as obtained by this model when there are recoveries is given by:

$$U=L_u = \frac{m p^n w^n a x}{r} - \frac{(v+M)}{a x},$$

and the corresponding expression of Macdonald's method is:

$$U=L_u = \frac{m p^n w^n a x^2}{r} - \frac{(v+M)}{a}.$$

We also found that h must be less than r for the occurrence of recoveries.

The critical level of mosquitos in relation to man is given by:

$$m = \frac{r(v+M)}{a^2 x^r p^n w},$$

which is higher than that obtained by Macdonald by the factor ' x '—the infective biting factor of mosquitos. This double representation of this factor is a necessary factor for the transmission of malaria. This may probably explain the phenomenon of anophelism without malaria.

The index of stability giving the minimum increase in the basic rate necessary to raise the endemic level L_u from 0 to 1 is given by ' IS ' = $[(ax)/(v+M)]$, thus, expressing directly the probability of an anopheline vector having an infective feeding on man during its life-time. The corresponding expression of Macdonald's theory is ' IS ' = $[(a)/(v)]$, expressing the probability of an anopheline vector having a feeding on man during its life-time.

We found as in Macdonald's theory that the index of sensitivity giving the minimum change in the endemic level due to a change in one of the controlling factors is the reciprocal of the index of stability. Perhaps these indices for the stability and sensitivity obtained here give better explanation for the occurrence of epidemics or outbreaks as these take into account the infective material in the community at a given time.

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FACTORS AFFECTING THE MOUSE PROTECTIVE VALUE OF CASEIN HYDROLYSATE PLAGUE VACCINE

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The error involved in the mouse protection test of plague vaccine was about 100 per cent. Giving the vaccine in two divided doses at an interval of one week produced 2½ times better immunity than when given in a single dose; challenging the mice one week after the second immunising dose gave the same results as challenging after two weeks. It is therefore evident that full immunity was developed within one week after giving the second dose of vaccine.

When plague pandemic broke out and Yersin (1894) and Kitasato (1894) had isolated the infective organism as *Yersinia pestis*, a number of workers carried out experiments to see whether vaccine made from this organism would give protection against infection. Many methods were used to prepare the vaccine and different animals such as rabbits, guinea-pigs, wild rats, monkeys, mice etc. were employed to determine whether the vaccine gave protection [Yersin, Calmette and Borrel (1895) Haffkine (1896) (Quoted by Bannerman 1902) Wyssakowitz and Zabalotony (1897), Gaffky, Pfeiffer, Sticker and Dieu-donne (1899) Kolle and Otto (1903, 1904) Strong (1907), Rowland (1910), Flu (1919, 1929), Stevenson and Kapadia (1924), Schutze (1925, 1932, 1939), Naidu and Jung (1929), Otten (1933) and others]. All these experiments were qualitative in nature. No efforts seem to have been made to find quantitatively the amount of vaccine needed to give protection and how one vaccine compared with another.

Sokhey and Maurice (1935) developed

a mouse protection test. They standardised the challenge dose which was constant both as regards the number and also the virulence of the organisms. They were thus able to get results which agreed very closely in repeat tests. The authors just described the method of assay and did not present the related aspects of the problems. It is the purpose of this paper to present some of these aspects.

Materials and Methods

I. *Error of the method of testing the vaccine by the mouse protection test*—Formalised casein hydrolysate plague vaccine was prepared, tested and evaluated by the methods reported in a previous paper (Habbu, Jhala and Sokhey, 1973).

To estimate the error of the method involved in the mouse protection test the same vaccine was tested and evaluated seven times on different days. The results obtained are given in Table 1.

II (a) *Effect of immunization with a single dose and two divided doses : Plague vaccine*—The cultures were grown in casein

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hydrolysate medium, Sokhey, Habbu and Bharucha (1950), at 28° C for two weeks and killed by adding 0·070 per cent formalin and preserved by adding 1·5 mg/per cent phenyl mercuric nitrate.

Immunization—Five groups of six mice each were immunised with graded doses of the above vaccine in two equal doses at an interval of one week. A similar series of mice were immunised with the total doses of the vaccine given to mice of first series in only one dose, on the day the first series of mice received the second dose of vaccine. Test infective dose was given to both the series a week after the second series of mice received the vaccine dose. The results obtained are given in Table II(a).

II (b) *Effect of period elapsing between the immunising dose and test infective dose on the mouse protective value of the vaccine*—To determine the effect of giving the test infective dose, two weeks after or one week after the second immunising dose, experiments similar to the above were carried out. Five groups of six mice each were immunised with graded doses of the above vaccine in two equal doses at an

interval of one week. A second series of mice received the first dose of the vaccine on the day the first series of mice received the second dose of vaccine. The second series of mice received the second dose of vaccine a week later and a week after that both the series of mice were given the challenge dose. Therefore, the first series of mice received the challenge dose 14 days after receiving the second dose of the vaccine and the second series, one week after the second vaccine dose. The results obtained are given in Table II(b).

Results

Results given in Table I show that the mouse protective doses of the same vaccine varied from 0·0017 ml to 0·0025 ml with a mean value of 0·0021 ml. The standard deviation was 0·00033 ml which gave the fiducial limits of 0·0014 ml and 0·0028 ml. The results of the assays were (1) fairly regular, (2) showed graded response with graded doses, (3) were repeatable, (4) the method was fairly sensitive. Hence the method could be depended upon to draw valid conclusions.

TABLE I

Results of seven repeat tests of casein hydrolysate vaccine No. 140 to show the error involved in the mouse protection test

Batch No. of vaccine	Dose of vaccine in ml					No. of organism in challenge dose	Controls	50% mouse protective dose in ml
	0·0005	0·001	0·002	0·004	0·008			
140	.. *6/6	4/6	2/6	3/6	0/6	128	12/12	0·0018
..	.. 6/6	5/6	5/6	1/6	1/6	108	12/12	0·0025
..	.. 5/6	5/6	2/6	1/6	1/6	170	6/6	0·0017
..	.. 6/6	5/6	4/6	2/6	0/6	136	6/6	0·0025
..	.. 6/6	5/6	2/6	3/6	0/6	164	6/6	0·002
..	.. 5/6	5/6	4/6	2/6	0/6	132	6/6	0·0024
..	.. 6/6	4/6	2/6	3/6	1/6	126	6/6	0·0021
						Mean value		0·0021
						Standard Deviation		0·00033
						Fiducial limits 0·00144 to		0·00276
						Deaths / Total		

Results given in Table II(a) showed that immunisation of mice with two equally divided doses gave about $2\frac{1}{2}$ times better immunity than that when given in a single dose.

TABLE II(a)

Immunity developed by giving the vaccine (1) in one dose, (2) in two equally divided doses

Expt. No.	50% mouse protective dose when vaccine was given in	
	One dose	Two equally divided doses
1	0.0080 ml	0.0048 ml
2	0.0075 ml	0.0036 ml
3	0.0120 ml	0.0032 ml
4	0.0130 ml	0.0036 ml
Mean value	0.0100 ml	0.0038 ml

Results given in Table II(b) showed that challenging immunised mice one week or

two weeks after the immunising dose did not produce any change in the mouse protective dose. Therefore, it is evident that full immunity was developed within one week after the second dose of the vaccine.

TABLE II(b)

Immunity developed by giving the challenge dose (1) one week and (2) two weeks after the second dose of vaccine

Expt. No.	50% mouse protective dose when challenge dose was given	
	One week after	Two weeks after
1	0.0070 ml	0.0066 ml
2	0.0070 ml	0.0076 ml
3	0.0060 ml	0.0072 ml
4	0.0080 ml	0.0071 ml
Mean value	0.0070 ml	0.0071 ml

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LIVER FUNCTION TEST IN RELATION TO HEPATITIS B ANTIGEN IN HEPATITIS, LEUKAEMIA AND LEPROSY

The association of Australia (Hepatitis B) antigen in relation to viral hepatitis, leukaemia and leprosy has been investigated. Abnormal liver function tests were associated with presence of Australia Antigen in viral hepatitis while no significant changes in liver function were evident in cases of leukaemia and leprosy. Findings are discussed in the light of available data.

JYOTI A. BELLARE*, K. V. KULKARNI* AND A. J. BAXI†

Blumberg(1) discovered an unusual antibody in the serum of haemophiliac patient who had received many transfusions; this reacted with an antigen in the serum of an Australian aborigine and so termed the Australia antigen(2). Subsequent studies demonstrated that Australia antigen [synonyms: hepatitis-associated antigen (HAA); serum hepatitis (SH) antigen, hepatitis-B-antigen (HB-Ag)] was associated with viral hepatitis (type B), Down's syndrome, various forms of leukaemia, and lepromatous leprosy(3, 4, 5, 6, 7, 8). The prevalence of HB-Ag in normal people was found to be low in the United States (0.5 per cent) but raised in tropical countries(4). This carrier state also occurred in a number of persons without a history of viral hepatitis. Acute viral hepatitis is associated with severe derangement of liver function. Little data are available on liver function tests in HB-Ag carriage in the other conditions. In patients with Down's syndrome liver function tests were found to be compatible with anicteric hepatitis(9). A study of liver function tests in patients with viral hepatitis, leukaemia and lepromatous

leprosy was therefore undertaken to assess the liver pathology as a result of parasitism by the hepatitis B virus.

Materials and Methods

Serum samples of patients suffering from viral hepatitis, leukaemia and leprosy were collected from the Kasturba Hospital for infectious diseases, Tata Memorial Hospital and Acworth Leprosy Home, Bombay. Details are given in Table I.

Liver function tests included serum transaminases (SGOT and SGPT) (10), alkaline phosphatase(11) and total bilirubin(12). HB-Ag was determined by counterimmunoelectrophoresis (CEP) (6).

Results and Discussion

Details of the liver function tests are presented in Table I.

Viral hepatitis.—SGOT and SGPT levels were higher in HB-Ag positive cases than in HB-Ag negative cases. This difference was not significant. This is in contrast to our earlier findings(6) where the SGPT levels were higher in the HB-Ag negative group. This apparent discrepancy can be

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explained on the basis of the stage of the disease. Most of the cases after being treated by the general practitioners enter the hospital in the late stage of the disease. At this stage HB-Ag probably disappears in many cases, and patients are recovering from clinical disease. Normally serum enzyme levels assume higher levels in infectious (HB-Ag negative) hepatitis. Thus these negative cases in the present series are those with transient HB antigenemia. Alkaline phosphatase and bilirubin levels did not show significant variation.

Leukaemia.—Liver function tests in leukaemia patients with and without HB-Ag showed no differences, when compared with the controls. No attempt has been made to categorise different types of leukaemia as the series was small. These findings are surprising in view of the fact that in Down's syndrome (who

have a higher incidence of leukaemia) some derangement of liver function is seen(9). The present studies, however are inadequate to draw firm conclusions and further indicate normal liver status.

Lepromatous leprosy.—SGOT and SGPT levels show an increase above the normal suggesting some disturbance of the liver. The inference we could draw is that carriage of HB-Ag is associated with damage to the liver. It would be interesting to examine the histological changes which could not be done in the present study.

Our studies indicate that the carriage of the hepatitis-B-virus in diseases other than viral hepatitis (type B) may not always lead to pathological changes in the liver. It may be that in viral hepatitis another infective agent is present(9) along with HB-Ag to produce a sequential pattern of liver pathology.

TABLE I

Liver Function Tests in various diseases in relation to HB-Ag

Liver Function Test	Name of the diseases							
	Normal	Viral Hepatitis		Leukaema		Leprosy		
		HB-Ag +	HB-Ag —	HB-Ag +	HB-Ag +	HB-Ag +	HB-Ag —	
SGOT (Karmen units)	21.2±1.40* (30)†	115.6±8.4 (22)	99.4±9.2 (39)	33.2±4.8 (8)	28.5±1.7 (44)	67.5±12.1 (8)	34.4±3.3 (20)	
SGPT (Karmen units)	25.7±1.80	159.9±13.4	127.0±10.5	31.4±5.0	23.7±1.7	62.2±10.0	19.2±1.5	
Alkaline Phosphatase (Bodansky units)	3.2±0.28	4.18±0.48	4.97±0.58	2.8±0.35	2.9±0.09	2.7±0.47	2.3±0.09	
Bilirubin (mg/100 mm)	0.28±0.01	6.54±0.32	8.60±1.1	0.37±0.04	0.32±0.07	0.5±0.19	0.5±0.09	

* Mean ± standard error.

† Figures in parentheses indicate No. of cases.

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LABORATORY TRIALS OF A NEW RODENTICIDE : RH 787

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Several new compounds are being evaluated for their rodenticidal properties all over the world. RH 787 showed a promise in initial tests as a rodenticide against a wide variety of rodents in the United States and London. The compound was evaluated against Indian species of rodents viz., *Rattus rattus*, *Rattus norvegicus*, *Bandicota bengalensis* and *Mus musculus* under laboratory conditions. The compound was found to be readily acceptable to rats and showed marked degree of efficacy.

Introduction

The control of rodents remains an insurmountable task even today.

RH 787 (N-3-pyridylmethyl-N'-p-nitrophenylurea), a compound developed by M/s. Rohm and Haas showed promise as a rodenticide in initial stages in U.S.A. and London(1). The compound was found to be effective against a wide variety of rats and was therefore submitted to us for laboratory evaluation against species of Indian rodents.

The findings are presented in the following account:

Materials and Methods

The trials with RH 787 were conducted against four Indian species of rodents, viz., *R. rattus*, *R. norvegicus*, *B. bengalensis* and *M. musculus*. Sexually mature, wild healthy animals were used for all the tests. Test animals were acclimated in test cages for one month before commencement of the experiments. Four days before the initiation of trials, these animals were maintained on basal ration. The composition

of basal ration is mentioned below:

Basal Ration

Cornmeal Crude	65 per cent
Steel Cut Oats	25 per cent
Powdered Sugar	5 per cent
Vegetable Oil	5 per cent

A set of test animals consisted of four individually caged, acclimated animals of each species. In all sixteen animals were used per test and four tests were performed for 2 per cent bait formulation four were performed with 1 per cent bait. Basal ration was used as the carrier bait material. Two feeding containers were used for each cage. One feeding container was filled with 30 gm of basal ration. The second container was filled with 30 gm of bait. The containers were anchored in feed positions within each cage. Water was offered *ad lib.* in water bottles and its consumption was recorded. Consumption of bait and basal ration was recorded every day and the bait was replenished to 30 gm level. Position of bait and basal ration in the cage was reversed daily. At the end of the third day the bait was discontinued and the survivors were maintained on water and basal

ration for additional five days observation period. Mortalities were recorded on the day of test they occur i.e., 1st, 2nd, etc. The period of mortality was worked out in hours. The test was terminated at the end of eight days. A second set of animals, consisting of four rodents of each species, i.e., sixteen in all was kept as control for each test. The control rodents were offered only basal ration. Their daily intake of water and food was recorded and mortality if any, in control rodents was noted.

Results

TABLE I

Mortality with 2 per cent bait formulation

Rat Species	Mean weight in gm	Percent Mortality	Mean period of Mortality (Hours)	Average poison consumption mg/kg
<i>R. rattus</i>	137.5	100.0	24.7	163.8
<i>R. norvegicus</i>	243.5	81.2	25.4	192.2
<i>B. bengalensis</i>	407.5	100.0	21.5	125.7
<i>M. musculus</i>	25	100.0	35.6	850

Table I indicates percentage mortality with 2.0 per cent bait formulation. With the exception of *R. norvegicus*, 100 per cent mortality was encountered in all the three species of rodents, viz., *R. rattus*, *B. bengalensis* and *M. musculus* albino at the end of three day feeding period. In the case of *R. norvegicus*, only 81.2 per cent mortality was noted. Mean period of mortality was found to be 24.7 hours in *R. rattus*, 25.4 hours in *R. norvegicus*, 21.5 hours in *B. bengalensis* and 35.6 hours in *M. musculus* albino. Average quantity of toxicant consumed per kg of body weight ranged from 125.7 mg/kg in *B. bengalensis* to 850 mg/kg in *M. musculus* albino.

TABLE II
Percent Bait Acceptance

Rat Species	Day		
	I	II	III
<i>R. rattus</i>	75.0	6.0	19.0
<i>R. norvegicus</i>	68.0	..	12.5
<i>B. bengalensis</i>	81.2	6.0	12.8
<i>M. musculus</i> (albino)	35.7	31.3	33.0

In Table II percentage acceptance of RH 787 bait by different species of rodents tested is shown. 75 per cent *R. rattus*, 68.0 per cent *R. norvegicus*, 81.2 per cent *B. bengalensis* and 35.7 per cent *M. musculus* accepted the bait formulation on the first day. Acceptance of bait formulation was 100 per cent in *R. rattus*, *B. bengalensis* and *M. musculus* albino. In *R. norvegicus*, only 80.5 per cent rodents accepted the bait.

All rodents accepting RH 787 bait formulation succumbed to the poison and there was no survival in rodents accepting the bait. The earliest period in which mortality was noticed after the acceptance of the bait by rodents was two hours. It was also noticed that a single dose of 2.0 gm of 2 per cent bait formulation was adequate to induce 100 per cent mortality. Repetition of tests with 1.0 per cent formulation showed that percentage mortality was 100 per cent in *R. rattus*, *B. bengalensis* and *M. musculus* albino and it was 75.0 in *R. norvegicus*. This indicates that the use of RH 787 at 1.0 level could prove equally useful.

Discussion

The characteristics of an ideal rodenticide include high degree of toxicity to rodents, a very ready acceptability, safety to non-target animals and failure to induce 'bait shyness' when sublethal doses are consumed. RH 787 has shown high degree

of efficacy against a wide variety rodents, viz., Norway Rat, Roof Rat, Cotton Rat, Rice-field Rat, Lesser Bandicota Rat, Deer Mouse and House Mouse(1). Tests conducted at the Haffkine Institute indicate that the compound is effective against the four types of Indian rodents, viz., *R. rattus*, *R. norvegicus*, *B. bengalensis* and *M. musculus*. It was also found to be readily acceptable to various species of rodents tested.

Apart from being highly effective, the compound appears to be target specific. Its toxicity to dogs, cats, birds and other pets is very low(1).

Acute dermal toxicity (LD_{50}) of RH 787 was found to be higher than 4 g/kg in rabbits. Acute inhalation LC_{50} to rats was also observed to be low (2.06 mg/L). The compound does not cause any local skin or eye irritation (1).

Many new compounds are being tested in the laboratories and in the field for rodenticidal properties. Some of these are Gophacide, α -chloralose and organic

phosphates (2). However, none of these compounds fulfils the requirements of an ideal rodenticide. The search for new rodenticide therefore continues. The WHO Scheme for the evaluation and testing of new insecticides now includes rodenticides and new compounds—submitted for the most part by industry—are being screened by a number of collaborative laboratories both in the laboratory and in the field. Judging from the performance in the laboratory, it appears that in RH 787, an answer to the need for an ideal rodenticide is found. To what extent it fulfils the hope will depend only on its performance in the field.

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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, Material 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 & S Livingstone Ltd., Edinburgh, p. 28.

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