Toxicological Study of Intravenous Metronidazole in Laboratory Animals

A THESIS

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By

SATISH JANARDAN RAJHANSA
B. V. Sc. & A. H.

Bombay Veterinary College
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INTRODUCTION

Metronidazole was synthesised by Rhone-Poulenc in France (1957). Cosar and Joulou (1959) found metronidazole (1,2-hydroxyethyl-2-methyl-5-nitroimidazole) to be active against *Trichomonas vaginalis* infection when administered orally. Therapy of vaginal trichomoniasis with metronidazole 200 mg, thrice a day for seven days by oral route proved very successful and therapeutic application of metronidazole for vaginal trichomoniasis gained popularity for its clinical success (Durel et al., 1959 and 1960). Soon after this, it was also shown to possess therapeutic efficacy against other infections viz. giardiasis and acute ulcerative gingivitis (Fowler, 1960; Shinn, 1962). However, its value in amoebiasis was not realised until several years later (Powell et al., 1966).

The use of metronidazole in control of anaerobic infections has been well established (Shinn et al., 1961).

Most of the reports regarding the pharmacological and toxicological evaluations of metronidazole were based on oral administration (Fluker, 1961; Lewis and Kenna, 1965). Pardanani et al. (1973) had used metronidazole in the form of retention enema.
Information on parenteral administration of metronidazole is very scanty. As metronidazole is not soluble in water and sparingly soluble in other solvents (British Pharmacopoeia, 1973) a suitable injectable preparation could not be readily formulated.

A parenteral preparation was felt necessary for therapeutic use in Trichomonas foetus infection (Meszaros et al. 1973). Eysyn and Phillips (1976) reported the usefulness of intravenous metronidazole, given alone or with antibiotics, in the treatment of severe anaerobic infections in 24 patients. The response observed was dramatic and also life saving. Metronidazole was also being used prophylactically in pre and post-operative therapy (Willis et al. 1975).

Parenteral route, especially intravenous route would be of great value in the administration of metronidazole as very high levels of serum concentrations of metronidazole could be achieved. Moreover, the absorption of chemotherapeutic agents in general by oral administration is not satisfactory, particularly in ruminant.

Information on the toxic effects of parenteral preparation of metronidazole is very scanty in literature. It was therefore felt necessary to study the toxic effects of parenteral metronidazole in laboratory animals.
Moreover, the parenteral route of drug administration has got unique advantages particularly in ruminants to attain better therapeutic levels. A parenteral preparation of metronidazole (Metrogyl) formulated by M/s Unique Pharmaceutical Labs., Bombay was selected for study. This preparation is to be administered intravenously. As the drug is expected to be administered for a period of 4 to 7 days in human beings monitoring haematological changes and liver function tests would be of great value in knowing the probable toxic effects of the drug on cardiovascular system and respiratory system. The data obtained from these experiments may throw some light on the possible hazards of this drug if introduced clinically in human beings and animals.
REVIEW OF LITERATURE

Metronidazole, a drug synthesised by Rhone-Poulenc (1957) in France was found to be systemically active against Trichomonas vaginalis infection when given by mouth (Cesar and Julou 1959). Later it was also shown to possess useful activity against other infections notably giardiasis and acute ulcerative gingivitis (Fowler, 1960; Shinn, 1962). However, its value in amoebiasis was not realised until several years later (Powell et al. 1966). Reports on the efficacy of metronidazole in control of anaerobic infections were ample (Shinn et al.).

1. Chemistry of Metronidazole

Structural formula of Metronidazole is

\[
\begin{align*}
\text{HC} & \quad \text{N} & \quad \text{C-CH}_3 \\
\text{O}_2 \text{N} & \quad \text{N} & \quad \text{CH}_2 - \text{CH}_2 \text{OH} \\
\text{C} & \quad \text{N} & \quad \text{CH}_2 - \text{CH}_2 \text{OH}
\end{align*}
\]

Metronidazole is 1-(2-hydroxyethyl)-2-methyl 5-nitroimidazole. It contains not less than 99% and
not more than 101% of C₆H₉N₃O₃ calculated with reference to dried substance. It is a white crystalline or white creamy crystalline powder, with slight odour, and bitter and saline taste. It is soluble in 100 parts of water, 200 parts of alcohol (95%) and 250 parts of chloroform and slightly soluble in solvent ether. It has melting point 159°C to 161°C (British Pharmacopoeia, 1973). The bactericidal activity of metronidazole was not significantly affected by pH changes within range of 5.5 to 8.0 (Ralph, 1978).

11. Assay of Metronidazole

Metronidazole can be assayed by titrating unknown mixture with 0.1 N perchloric acid adding one drop of malachite green T.S. as an indicator. Titrate till yellow-green end point. Each one milliliter of perchloric acid (0.1 N) is equivalent to 17.12 mg. of metronidazole (United State Pharmacopoeia XIX).

Evans and Phillips (1976) bioassayed metronidazole in serum by plate method. Lysed blood agar plates were flooded with suspension of B. fragilis. NCTC 9343 and standard solutions of metronidazole were made in doubling dilution of horse serum standard and patients' sera were placed in well cut in the plates,
which were then incubated overnight in gas pack jars.

iii. Pharmacology of Metronidazole

1. Absorption:

Metronidazole is actively absorbed from small intestine and peak levels were achieved within one hour (David, 1978). Following rectal administration of 1 G. metronidazole the serum levels were 6.3 mcg/ml. after 2 hours and 11.0 mcg/ml. after 6 hours (Pardanani et al. 1978).

These levels were well above those required for control of anaerobic infections found in cases of intestinal perforation. Studies with metronidazole retention enema, 2 G in 200 ml. of saline have been reported and blood level of metronidazole achieved was 19.1 mcg/ml. at end of 12 hours (Olumide and Adesola, 1978).

ii. Distribution:

High concentrations of labelled drug were identified in liver, kidney, stomach and intestines at various time intervals after intravenous injections. The drug also reached the brain in unchanged form showing that it passes through blood brain
barrier (Placid et al. 1971). Concentration of 6.0 to 22.7 mcg/ml. in cerebrospinal fluid was equivalent to an average of 43% of simultaneous plasma concentration after single oral dose of 2.4 g. metronidazole in 4 healthy volunteers (Jokipii and Jokipii 1974). Metronidazole readily passed into placenta (Rea, 1977) and the drug also appeared in breast milk (Gray et al. 1961). Metronidazole and its metabolites reached the foetuses in pregnant females, but did not accumulate there (Placid et al. 1971). High concentrations had been found in serum, cerebrospinal fluid and in pus aspirated from amoebic liver abscess 48 hours after starting treatment (Kane et al. 1961; Cosar et al. 1962; Davies, 1967; Mcfadzean, 1969). Peak Plasma concentrations of 13.1 and 13.9 mcg/ml. respectively after oral and intravenous administration of single doses of 500 mg. were reported by Houghton et al. (1978).

Eykyn and Phillips (1976) had reported certain advantages of intravenous metronidazole, given alone or with antibiotics. (Intravenous metronidazole administered as buffered, isotonic 0.5% w/v aqueous solution in 100 ml. bottle containing 500 mg. metronidazole and infused every
3 hours over about 20 minutes). Reduction in dosages in cases of renal failure was not warranted. Serum samples were collected before infusion was started, 20 minutes after end of infusion and 48 hours after infusion. Mean serum concentrations were 6.4 to 26 mcg/ml and peak mean values were 11.5 to 41 mcg/ml.

Benggose and Barbammi (1973) had reported interaction of metronidazole with human and bovine, plasma albumin. The binding of metronidazole involved both hydrogen and hydrophobic bonds and at three sites. The drug was bound very strongly and depending upon dose, relatively small amount might be available in plasma to achieve a therapeutic effect. It was, therefore, possible that low systemic concentration of drug in circulation was due to its high affinity for plasma proteins. But Ralph et al. (1974) reported that metronidazole was only slightly bound (1 to 8%) to human plasma protein at therapeutic concentration. A value of 20% was reported by Amon et al. (1977 and 1978).

The elimination half life of oral metronidazole varied between 6.2 to 11.5 hours (McGilveray et al. 1973; Melander et al. 1977; Ralph and Kirby, 1977; Schwartz and Jeunet, 1976; Wood and Morno, 1975), but appeared not to be dose dependent (Weiling and Morno 1972).
After single intravenous dose (Oppermann et al. 1978) the half life of C \textsuperscript{14} - metronidazole was 7.7 hours and the total radioactivity 11.9 hours (Oppermann et al. 1978). The prolonged half life probably results from low renal clearance of 10.2 \text{ml/Min/1.73 m}^2 (Ralph et al. 1974).

iii. Metabolism:

Metronidazole eliminated largely as metabolic product from side chain oxidation, hydroxylation or conjugation of the parent compound (De Carneri et al. 1969; Stambaugh et al. 1968). The major metabolic product is 1-(2-hydroxyethyl)-3-hydroxymethyl-5-nitroimidazole along with its glucuronide accounts for 40 to 50% of recovered urinary material (Stambaugh et al. 1968). Unchanged metronidazole and its conjugates accounted for about 30% of recovered products (Stambaugh et al. 1968).

iv. Excretion:

Bulk of the compound is excreted in urine unchanged, although two oxidation products, which were less active than metronidazole against Entamoeba histolytica were isolated. It is excreted through bile and urine which later sometimes acquires reddish colour. Small amounts are also excreted through saliva,
semen, and vaginal secretions etc. (Sheth, 1973). The major metabolic products along with its glucuronide component are excreted in urine to the extent of 40 to 50% and unchanged metronidazole accounted for about 30% (Stambaugh et al., 1968).

v. Mechanism of Action:

Mechanism of action of metronidazole is not yet clearly understood. The action of metronidazole on protozoa was reported by Edward and Mathison (1970). According to them metronidazole inhibited in the evolution of hydrogen gas in Trichomonas vaginalis before it inhibited carbon dioxide evolution. A phosphoroclastic reaction of elastidic type was the major mechanism of action by which both gases were evolved. It seemed that metronidazole inhibited directly or indirectly the hydrogenase component of the system.

The mechanism of action of metronidazole on bacteria was reported to be due to inhibition of DNA synthesis (Frank, 1976). Metronidazole enters bacterial cell more readily under anaerobic conditions (Muller and Lindmark, 1975) and after reduction by some means to a more polar metabolite, possibly by low redox potential electron transport protein such as ferredoxin (Edward et al., 1973; O'Brien).
and Morris, 1972) binds to and degrades DNA (Chien and Mizuba, 1978; Edward et al., 1978). Nevertheless, recent studies have shown that reduction is essential for antimicrobial activity (Edward, 1977). Preliminary work by Tally et al. (1978) indicates that drug is taken up by both susceptible and resistant organism (but at greater rate by susceptible bacteria), but only the susceptible bacteria seem capable of metabolising drug. Degradation of existing DNA from exponentially growing cultures of C. bifurcans was noted by Plant and Edwards (1976) and Edward et al. (1978) but no degradation of mammalian, pneumococcal or T phage DNA could be demonstrated by La Russo et al. (1978).

vi. Effect of Gastrointestinal Tract

Botero (1978) had reported that approximately 30% of patients showed some signs and symptoms of intolerance, usually of low intensity. They are mainly restricted to gastrointestinal tract such as nausea, vomiting, abdominal pain, metallic taste and diarrhea.

viii. Effect on Central Nervous System:

Metronidazole 150-225 mg/kg. daily produced marked central nervous system effects in dogs, but there was no evidence of any morphological changes (Boat, 1977). Botero (1978) reported dizziness, joint and muscle pain, headache and numbness in 30% of the patients receiving metronidazole. Peripheral neuropathy was reported in a few
patients treated with oral or intravenous metronidazole for anaerobic infection ( Hunt et al. 1978 ).

viii. Effect on Haemopoietic system

Structurally related chemicals have caused blood dyscrasias, but serious difficulties have not been recorded with metronidazole. However, appreciable neutropenia in treated individuals was observed. However, it was reversible after course of medication was completed ( Lefebvre and Hesseltine, 1965). Intravenous metronidazole at dosages 60, 150 and 300 mg/kg, daily for 4 weeks did not produce any changes in haematological values ( Bost, 1977 ).

ix) Effect on Cardiovascular system:

Larger doses in experimental animals affected neither cardiovascular system nor respiration ( Powell, 1967). Intravenous metronidazole at dosages 60, 150 and 350 mg/kg daily for 4 weeks in rat did not produce any significant changes in Blood Pressure ( Bost, 1977 ).

IV. TOXICITY OF METRONIDAZOLE

1. Acute Toxicity:

Attempted suicide by ingestion of 4.2 gms ( Fluker, 1961 ), 3.6 and 12 gms ( Lewis and Kenna, 1969 ) resulted in minimal disturbances in human cases.
As reported by Hume et al. (1969) like quinine, primaquine and pentaquine, metronidazole also passes placental barrier, but there is little information regarding the fetal effect of such transfer, though one could predict hemolytic consequences of glucose-6-phosphate dehydrogenase.

11. Sub-acute and Chronic Toxicity:

The toxicity of metronidazole during long term administration varies between strains of mice and different animal species. High dosages (600mg/kg/day) caused weight loss and testicular dystrophy in one strain of mice but not in another (Bost, 1977). In rats high doses of oral metronidazole caused testicular dystrophy and decreased the rate of body gain. Intravenous metronidazole at dosages 60, 150 and 300 mg/kg daily for 4 weeks did not influence body weight gain nor produce any significant changes in blood pressure, haematology and in biochemical values. Sub-acute and chronic studies in the monkey with oral metronidazole (45 to 240 mg/kg/day) revealed a drug related effect on liver, evidenced by histological examination, without associated changes in serum enzyme levels (Bost, 1977). Metronidazole 150-225 mg/kg daily produced marked central nervous system effects in dogs, but there was no evidence of any morphological changes (Bost, 1977).
iii. **Dysmorphology studies:**

In rat, rabbit, mouse and guinea pig, administration of metronidazole at dosages considerably higher than the human therapeutic doses before and/or during pregnancy generally produced no dysmorphogenic changes (Bost, 1977).

iv. **Mutagenicity and Tumourigenicity of metronidazole:**

Metronidazole can be activated by mammalian tissues (human and laboratory animals) to substances mutagenic to *Salmonella typhimurium* (Rosenkranz and Speck 1977), but evidence (Lindmark and Muller, 1975) suggested that reductive biotransformation is essential for this effect. Hypoxic tumour cells have been only mammalian cells shown to provide environment necessary for this reduction (Baines, 1978). Dosages up to 1000 mg/kg daily for 5 weeks did not produce a dominant lethal effect in the mouse. Studies in rats given 300–600 mg/kg daily were less conclusive (Bost, 1977).

In the human fibroblast cells, metronidazole at levels up to 1400 mcg/ml did not significantly increase un-schedule DNA synthesis (Bost, 1977).

**Swiss mice fed high doses of metronidazole** (diet concentration up to 0.5%) for their lifetime showed
an increased incidence of lung tumours in males and females and malignant lymphoma in females only, but no increase incidence of lung tumour of other kinds (Rustia and Shubik, 1972). About 20% of control animals developed lung tumours and malignant lymphoma (Cohen et al. 1973) failed to find any significant difference between incidence of tumour development in control and those given metronidazole 0.135% for 66 weeks.

Hamster fed 0.3 or 0.15% metronidazole (Rustia, cited in Roe, 1977) or 30 or 80 mg/kg/day (Lowe and Ingham cited in Roe, 1977) from 7 to 8 weeks of age until latter did not exhibit any significant increase in tumour formation. Recent studies in experimental animal models (Jorgensen and Rushbrook, 1977; Mitchell, 1976) found no evidence of tumourigenecity and many such studies conducted at Mayo Clinic (Beard et al. 1978) revealed similar results, though not conclusive.

Many mutagenic and carcinogenic agents have been shown to induce unscheduled DNA synthesis in vitro and to produce heritable mutagenic events when ingested repeatedly over an extended period, but in studies conducted in Stanford Research Institute, metronidazole did not induce unscheduled DNA synthesis in vitro and was not mutagenic in male mice when given 190, 375 and 750 mg/kg/day for 8 weeks as described in the heritable translocation procedure (Brodgen et al. 1978).
V. CHEMOTHERAPEUTIC ACTION OF METRONIDAZOLE

i. On Protozoa:

The growth of intestinal protozoa in vitro was inhibited by metronidazole in following concentrations - (Powell, 1968):

1) *Trichomonas vaginalis* - 0.25 μg/ml.
2) *Trichomonas hominis* - 0.50 μg/ml.
3) *Entamoeba histolytica* - 0.25 μg/ml.
4) *Balamuthia coli* - 4 - 8 μg/ml.

It was also shown to possess useful activity against other infections notably giardiasis, and acute ulcerative gingivitis (Fowler, 1960; Shinn et al. 1962). However, its value in amoebiasis was not realised until several years later (Powell et al. 1966).

ii. On bacteria:

Metronidazole was first used in treatment of anaerobiosis by Shinn et al. (1963) in cases of acute ulcerative gingivitis. It has been shown that 8 μg/ml. level of serum was more than adequate to control anaerobic infection. Ralph et al. (1974) showed that minimum inhibitory concentration of metronidazole in 51 strains of anaerobic organisms was 6.2 μg/ml. Metronidazole was
active against Clostridium perfringens and Clostridium welchii in vitro at a concentration of less than 4 mcg/ml. (Chow et al. 1974; Dornbusch et al. 1975; Freeman et al. 1968; Fazi and Caukas, 1970). Many strains of Actinomyces spp. were resistant to metronidazole. Laboratory studies undertaken by Davies et al. (1964) revealed that the drug was highly effective against certain species of anaerobic bacteria and its possible value in prevention and treatment of tetanus and gas gangrene was postulated by Freeman et al. (1968). Tally et al. (1972) reported successful use of metronidazole in the treatment of Bacteroides infection.

The exact mechanism of pathogenicity of non-sporing anaerobiosis is not very well understood. Inhibition of phagocytosis of some bacteria in vitro has been reported recently by Ingham et al. (1977). Though metronidazole was highly effective against anaerobic non-sporing Gram-negative bacilli, anaerobic cocci and Clostridia, it was less effective against anaerobic non-sporing Gram-positive bacilli (Chow et al. 1975).

Metronidazole has no activity against aerobic bacteria. Some bacteria like Proteus, Pseudomonas and most of strains of E.coli were capable of inactivating metronidazole. This might explain the failure of drug to eradicate completely Entamoeba histolytica (Mc Fadzean, 1969).
Regarding the action of metronidazole on aerobes, an interesting observation was made by Willis and his colleagues in their studies (1974, 1976 and 1977). It was shown that metronidazole prophylactically reduced not only anaerobic but also aerobic post operative infections in appendicular surgery (Willis et al. 1976), large bowel surgery (Willis et al. 1974). In this connection studies of Ingham et al. (1977) were highly significant. In in vitro study they demonstrated that obligate anaerobes interfered with phagocytosis and killing of Proteus mirabilis and other aerobic bacteria and that this effect was highest with strains of B. melaninogenicus and B. fragilis. It was suggested by Ingham et al. (1977) that by their inhibitory effect on phagocytes the anaerobes might be providing a certain degree of transient protection to the aerobes leading there by to establishment of aerobic infections.

Pre-operative administration of metronidazole reduced the number of anaerobes and remove this inhibitory effect on phagocytes. Under these circumstances the phagocytes can effectively deal with the aerobes which might explain the reduction of post operative infection by aerobes described by Willis and co-workers (loc.cit.).

The major antimicrobial agents which are useful in the treatment of anaerobic infections are penicillins, cephalosporins, tetracyclines, chloramphenicol, lincomycin,
clindamycin and metronidazole. *Bacteroides fragilis* is the most commonly encountered anaerobe, is also the most resistant to antimicrobial agents. The choice of antimicrobial agents, therefore will largely depend upon not only the infecting organism but also on the pharmacological properties of various drugs, their toxicity and the impact on normal flora and on their bactericidal action. Each one of the antibiotics has some disadvantages which may be specific viz. the bone marrow toxicity of chloramphenicol, the failure of lincomycin and clindamycin to pass the blood brain barrier and their tendency to cause pseudomembranous colitis or the disadvantages may be general in that these antibiotics are not consistently bactericidal at concentration readily available in serum or at site of infection. On other hand, available published report indicates that metronidazole which has been used widely in treatment of trichomoniasis, giardiasis, amoebiasis, dracunculiasis and ulcerative gingivitis does not appear to have any of these effects.

Today metronidazole has taken its place alongside chloramphenicol, lincomycin & clindamycin in treatment of anaerobic infection. Metronidazole is compatible with commonly used antibacterial antibiotics as reported by Salem et al. (1975). A study conducted by Salem et al. (1975) in which metronidazole in concentration up to 50 mcg/ml.
was combined with 14 antimicrobial agents commonly used in treatment of infections caused by aerobic and facultative bacteria, showed that there was no interference with activity of these agents against Staphylococcus aureus, Escherichia coli or Proteus mirabilis. Similarly, in studies Bacteroides fragilis there was no antagonism between metronidazole and clindamycin, erythromycin, carbenicillin, cefoxitin (Busch et al. 1976), spectinomycin (Wise et al. 1975), spiramycin, rifampicin, nitrofurantoin (Salem et al. 1975) or bencylpenicillin (Denner et al. 1969).

iii. Therapeutic Uses.

Cosar and Julou (1959) found metronidazole to be systemically active against Trichomonas foetus infection when given by mouth. Later it was also shown to possess useful activity against other infections notably giardiasis and acute ulcerative gingivitis (Fowler, 1960; Shinn et al. 1962). However, its value in amoebiasis was not realised until several years later (Powell, 1965).

In animal field, the uses of metronidazole were reported since 1961. Mandow et al. (1961) reported cure from giardiasis, trichomoniasis, and amoebiasis in mice with 266 mg/kg. body weight for eleven days without any toxic effect.

Luthgen and Beranau (1967)
proved efficacy of 10 mg/kg. body weight metronidazole for five days by giving injection in crops or in drinking water in treatment of pigeon trichomoniasis. Actor et al. (1969) have reported the emergence of resistance of *Trichomonas vaginalis* in hamster infected intravaginally after sub-therapeutic doses (50 mg/kg) of metronidazole. Meszaros et al. (1973) reported use of this drug in local treatment of *Trichomonas foetus* and found to be more effective than furazolidone and trypanflavine. He had suggested use of metronidazole in chronic infection with localization of parasite in urinary tract and accessory tract of genital system of bulls and for this purpose intravenous metronidazole therapy was desirable.

In Veterinary field, particularly in dogs Dean et al. (1969) recorded treatment of ulcerative gingivitis with metronidazole. Condition was similar to Vincent's gingivitis in human being as described by Chawla and Mathur (1969). Shankar et al. (1977) reported use of metronidazole in ulcerative gingivitis in dogs.

Ulcerative gingivitis mainly caused by *B. fusiformis* and *Spirochetes* was quite common in neglected dogs. According to him this drug was fairly satisfactory except in three cases which were associated with vomiting, nausea, retching and uneasiness.
Reshetnyak et al. (1970) reported use of metronidazole in treatment of rabbit coccidiosis. Spontaneous coccidiosis was treated by metronidazole at a dose rate of 40 mg/kg body weight orally for three days and also sub-cutaneous injection of 1% metronidazole at 20 mg/kg body weight. The metronidazole controlled both hepatic and intestinal coccidiosis in rabbits.

Kurpenko (1970) reported the use of metronidazole prophylactically in Balantidium infection in pig at a dose level of 0.25 g. per pig given twice daily for three days. Rao (1973) found trophozoites of Balantidium coli on examination of 24 cattles and buffaloes which were suffering from diarrhoea. He had shown that treatment with metronidazole (200 mg. per day for 4 days) cured 64 out of 84 cases. Therapeutic value of nitroimidazole derivatives in enteropathies of piglet intensive unit was studied by Negru (1972). The result of administration of metronidazole to 1589 piglets, in a unit where Balantidium coli infection and enteritis were prevalent, showed encouraging results with dose range of 0.013 - 0.039 /kg. body weight in six doses. Faecal samples were negative for Balantidium coli and Trichomonas from 24-68 hours after commencement of treatment and good clinical improvement was observed.

Borzemska and Zajac (1973) reported good improvement with metronidazole administered in drinking water to chicks during outbreaks of histomoniasis.
Recently Clare (1977) used metronidazole pre- and post-operatively in a dog for the removal of melanoma and to his surprise he found that healing of ulcer was satisfactory and secondary tumour remained static for four months.

Metronidazole was first used in treatment of anaerobiasis by Shinn et al. (1962) in cases of acute ulcerative gingivitis. Since then newer therapeutic use of metronidazole to control anaerobic infection were established.

According to Finegold et al. (1975) following were the major anaerobes encountered clinically,

1) Gram positive cocci vis. Peptococci and Peptostreptococci

2) Clostridia

3) Non-sporing Gram negative bacilli (NSGNB) vis. Bacteroides and Fusobacteria.

4) Non sporing Gram positive bacilli (NSGBP) vis. Actinomyces arachnia, Eubacterium and Bifidobacterium species.

Reports have appeared in literature in the last few years on the value of metronidazole in the treatment of various anaerobic infections including septicaemia,
necrotizing pneumonia and pulmonary abscess (Nitre and Rotheram 1974, Tally et al. 1972), post hysterectomy, pelvic cellulitis anaerobic intra-uterine infections (Willis and study group 1974), brain abscess (Ingham et al. 1975) and breast abscess (Hale et al. 1975).

Pre and post-operative oral treatment with metronidazole greatly reduced the incidence of post-operative non-clostridial anaerobic infections after hysterecmy (Willis and study group, 1975). The prophylactic and therapeutic value of metronidazole in appendicular surgery, elective colonic surgery and colorectal surgery have also been documented (Willis et al. 1976, 1977; Goldring et al. 1976 and Feathers et al. 1977).

Jackson and Golden (1976) had shown basic difference between cow's milk and human milk. According to them cow's milk was to promote bacterial growth in upper bowel, whereas human milk has evolved to discourage bacterial growth. Some malnourished children when fed cow's milk, tend to develop rumen like condition and this then referred as cow's milk protein allergy. Such cases were treated with 100 mg. metronidazole twice a day, for five days which acted specifically on anaerobic infection. Dramatic results have been obtained and after treatment children previously intolerant to cow's milk were able to consume normal quantities of cow's milk without any symptoms.
From above cited examples metronidazole has proved its value as an antibacterial in control of anaerobic infection along with its value as an antiprotozoan disease.

vii. INTERACTIONS OF METRONIDAZOLE WITH OTHER DRUGS

Alcohol is contraindicated with metronidazole because it produces disulfiram like reaction, characterised by confusional state, headache, flushing, nausea, vomiting, drowsiness and fall in blood pressure. These symptoms appeared as a consequence of inhibition of enzyme concerned with metabolism of alcohol (Botero 1978). However, disulfiram like reaction did not occur in all patients who drink alcohol while receiving metronidazole (Rothstein and Clancy, 1969).

A clinically significant interaction between warfarin and metronidazole was reported by Kranzner (1976) in a patient whose prothrombin time had previously been satisfactorily controlled on warfarin over a period of six years.

Dosage Schedules of Metronidazole:

Common route of administration of metronidazole is oral. Dose for trichomoniasis is 600 mg. in divided doses for seven days and for intestinal and hepatic amebiasis is
1.2 to 2.4 gms. daily for 5 - 10 days
(British Pharmacopoeia, 1973).

In the treatment of anaerobic infection, the usual adult oral dosages is 400 mg., 3 times daily for 7 days or longer according to clinical response. The oral dosages in infant, children is 7.5 mg/kg. 3 times daily. If rectal suspension are to be used, adult should receive 1gm., 3 times daily for 3 days and then 12 hourly for 4 days, the corresponding children's (5 to 12 years) dose begin 0.5 gm. administered as for adult younger children should receive 250 mg. doses for 1 to 5 years old and 125 mg. doses for those under one year. Oral medication should be resumed as soon as possible (Brodgen et al. 1978).

By intravenous infusion, the usual dosages for adults in 500 mg. in 100 ml. solution, 8 hourly, administered at a rate of 5 ml. per minute. For children under 12 years the intravenous dosage 7.5 mg/kg. infused as per adult (Brodgen et al. 1978).

In the prevention of anaerobic infection in gynaecological surgery, 1g orally as single dose followed when possible 200 mg. orally, 3 times a day upto 7 days after surgery, is recommended regimen. In elective colonic surgery metronidazole has been successfully used when given
alone (lg. orally as a single dose, 200 mg. 8 hourly thereafter when possible and lg. rectally, 8 hourly during period when oral medication is not possible, for total 7 days treatment) and when given concomitantly with Kanamycin or phthalysulfathiazole (Brodgen et al. 1978).
MATERIAL AND METHODS

The present study was undertaken to study probable toxic effects of metronidazole when given by intravenous route. Intravenous metronidazole infusion (350 ml. bottle) containing metronidazole I.P. 2 mg/ml. and dextrose I.P. 5% W/V and another intravenous infusion containing 500 mg. metronidazole I.P. in 100 ml. bottle containing water for injection q.s. were undertaken for study. The drug formulations were manufactured and supplied by M/s. Unique Pharmaceutical Labs., Worli, Bombay. The following parameters were selected for toxicological study -

1. Pyrogen Test (Rabbits).
2. Acute Toxicity study (LD 50) in mice.
3. Effect on cardiovascular system and respiratory system of dog.
4. Effect on haematology of dogs (21 days study).
5. Effect on Serum-alanine-aminotransferase (SGPT) and Serum-aspartate-aminotransferase (SGOT) enzyme in dogs.
1. **Pyrogen Test**

Pyrogen test was carried out as described in Indian Pharmacopoeia 1955 and supplement 1975 to Indian Pharmacopoeia, 1966.

Three healthy rabbits weighing more than 1.5 kg. were selected for the test. These rabbits were not utilised for any test for last two weeks. Each rabbit was housed in a separate cage in the animal house. They were also protected from any disturbances which would cause excitement. They were kept on uniform unrestricted diet and water for one week. Their initial weights and weights after one week were recorded to assess their general health. By using centigrade thermometer four rectal temperatures at 2 hour interval were recorded as control study.

The weights of rabbits were recorded before starting the experiment. The initial temperatures of the rabbits were recorded. None of them showed temperature less than 38°C and more than 39.5°C. The day prior to test, all glass syringes (50 ml.) and needles (23 G and 24 G) were autoclaved under 15 lbs/sq.inch pressure for 20 minutes.
On the day of test, three observations at half an hour interval were recorded prior to injection. Solutions to be tested for pyrogen test contained 2 mg/ml. metronidazole I.P. and Dextrose 5% W/V was warmed to 37°C approximately. The dose of 10 ml. per kg. body weight was injected intravenously by using marginal ear vein of the rabbit within 15 minutes subsequent to the normal temperature recording on the test day. Temperatures were recorded thereafter at a half an hour interval for three hours. Thereafter the rabbits were given unrestricted feed and water ad lib.

ii. **Acute Toxicity Test in Mice.**

Acute toxicity test of injectable metronidazole by intravenous route in mice was conducted. The mice of strain NIH-Swiss albino, weighing between 20 to 22 gms. supplied by Haffkine Biopharmaceutical Corporation, Bombay were used.

The animals were divided into six groups, each group contained sixteen mice (8 males and
Above preparation was so diluted with normal saline, so that each group will get concentration of metronidazole at the rate of 50 mg/kg., 100 mg/kg., 150 mg/kg., 200 mg/kg., in first four groups respectively. The fifth group had received preparation as much so that they will get concentration 250 mg/kg. Sixth group was served as control and they received only normal saline. Prior to day of test, tuberculin syringes and needles (26 G) were sterilized by autoclaving under 15 lbs/sq.inch for 20 minutes.

On the day of test, mice were given only ad lib water but no feed. The metronidazole preparation was injected in caudal vein of mice. Xylol was applied locally for raising caudal vein. Total volume of injection per mouse was one ml.

After injection mice in all four groups were observed for 24 hours as well as subsequent 6 days. After 24 hours, they were offered routine unrestricted feed.
111. Effect of Metronidazole on Cardiovascular System and Respiratory System of Dog.

The experiments were conducted as described by Sheth et al. (1972). The dogs weighing more than 10 kg. were anaesthetised with Nembutal (Sodium-pentobarbitone) at the rate of 35 mg/kg. body weight intravenously into saphenous vein. Sodium pentobarbitone was prepared freshly in normal saline at the concentration 70 mg/ml. Dogs were given Chlorpromazine hydrochloride at the rate of 1 mg/kg. intravenously as a preanaesthetic.

Anesthesia was achieved in about 60-90 seconds. Femoral vein, in femoral triangle, was cannulated and connected to normal saline 500 ml. bottle by means of dropper control. Normal saline was administered at the rate of 10-15 drops/minute.

Tracheotomy was also performed and cannula was inserted which was then connected to tambour by means of the pressure rubber tubes for recording respiration. Carotid artery was also cannulated with arterial dog cannula and was connected to mercury manometer for recording blood pressure. Kymograph was put on and
continuous recording of dog blood pressure and respiration were performed. Responses for vasopressor drug such as non-epinephrine and vaso-depressor drug such as acetylcholine were recorded.

The normal saline was replaced by metronidazole injection (having 350 ml. capacity bottles containing metronidazole I.P. 2 mg./ml. and 5% Dextrose I.P. w/v). The infusion of metronidazole was continued for an hour during which continuous recording was done. Then again vaso-pressor and vaso-depressor drugs were repeated in the same dosages to see any blocking or potentiation effect due to metronidazole. Experiment was terminated by giving intravenous saturated magnesium sulfate solution.

With 5 mg/ml. of metronidazole I.P. in 100 ml. bottle dog blood pressure, respiration and ECG were recorded on four channel Plywrite (INCO. Ambala). Blood pressure was calibrated at 75 mm Hg. per one centimeter. Responses for vaso-pressor such as non-epinephrine and vaso-depressor such as acetyl choline were recorded during normal saline infusion. This was then replaced by 100 ml. bottle containing 500 mg. of metronidazole I.P. Rate of flow was controlled to 5 ml/min. Continuous recording was done for 10 minutes and then the same dosages of the vaso-pressor and vaso-depressor drug such as non-epinephrine and acetylcholine.
were repeated. The experiment was terminated as mentioned previously by giving intravenous saturated magnesium sulfate solution.

iv. **Effect on Haematology of Dogs.**

Haematological studies were made on six mongrel dogs of either sex. For the present experiment, 6 apparently healthy dogs of approximately same age, were selected from Dog Destruction Centre, Mahalaxmi. Dogs were weighing between 13 to 16 kgs. Uniform feeding regimen was followed and continued throughout the experimental period. They were maintained under similar environmental conditions. Faecal samples and urine samples were collected and examined to assess general health of the animals and to detect, if any, parasitic infestation. For faecal examination, sedimentation procedure was used. It was found that all faecal samples were contained eggs of *Ancylostoma caninum* and only three dogs were found to have eggs of *Spirocerca lutea*. So all dogs were given 'Ancylo' at the rate of 1 ml/4.5 kg. body weight, subcutaneously. As dogs were housed in the new environment, they were under observation for fourteen days for acclimatisation in the new environment.
On the day prior to test blood samples of all dogs were collected. From the next day, each dog received intravenous infusion of 500 mg. metronidazole I.P. in 100 ml. over a period of twenty minutes. This infusion was given daily for seven days.

On 7th day and subsequently on 14th and 21st day blood samples were collected. Saphenous and femoral veins were used for intravenous infusion as well as collection of blood. As infusion was to be given for long time, after every infusion or collection of blood, 'Beparin' ointment was applied locally at the site of vein puncture to minimise phlebitis due to repeated punctures.

METHODS FOR ESTIMATION OF HAEMOGRAM

Haemogram was studied immediately after collection of blood.

1. Collection of Blood:

Blood was collected from saphenous vein in an empty 5 ml. vial containing oxalate as anticoagulant oxalate. The blood smears were prepared by taking fresh drop of blood from punctured vein on a clean glass slide so as to get thin blood film.
Dry syringes into which one drop of an anticoagulant was placed and allowed to coat inside the syringe. Care was taken in obtaining blood samples by syringe, as the use of too much vacuum might cause distortion of cellular elements.

**Anticoagulant**

The oxalate preparation of Heller and Paul (1934) was used. The stock solution of oxalate mixture contained 1.2 gm. of ammonium oxalate and 0.8 gm. of potassium oxalate dissolved in 100 ml. of water. 0.5 ml. of this mixture was placed in each vial and allowed to dry in hot air oven at 60°C. This quantity of anticoagulant was sufficient to prevent coagulation of 5 ml. of blood. Oxalate is an inexpensive anticoagulant that was easy to prepare. Either of these salts alone, was not recommended as a ammonium oxalate produces an increase in cell size and potassium oxalate decreases. Hence, combination of these salts was recommended.

**Estimation of haemoglobin**

Haemoglobin was estimated by using Sahlis haemoglobinometer. Reading was taken when colour was just matching with the comparator, supplied with
hemoglobinometer. The column of brown colour was measured with the help of scale graduated on the tube. This value gives the haemoglobin in gram per 100 ml. of blood.

Erythrocyte Count

Erythrocyte count was made by diluting the blood 1/200 by R.B.C. diluting fluid. Hayem's solution was used for diluting RBC with following formula.

<table>
<thead>
<tr>
<th>Mercuric Chloride</th>
<th>0.5 gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Sulfate</td>
<td>5.0 gm.</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>1.0 gm.</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>200.0 ml</td>
</tr>
</tbody>
</table>

RBC count was made with the help of Haemocytometer containing improved Neubauer's chamber and as per method described by Coles (1974), two counts were made for each blood sample and their mean was taken for calculating RBC count.

Following formula was used for total RBC count.

Total RBC count per cubic millimeter of blood = Total number of RBC in 5 squares x 10,000.
Total Leukocyte Count

The blood was diluted \(1/20\) with WBC diluting fluid having the following formula.

- Glacial acetic acid 2.0 ml.
- Gentian Violet 1% 1.0 ml.
- Distilled Water 100.0 ml.

The WBC count was made as per method described by Coles (1974) with help of Hemocytometer containing improved Neubauer's chamber.

Following formula was used for total leukocyte count:

Total leukocyte count per cubic millimeter of blood

\[
\text{Number of leukocytes} = \frac{\text{No. of leukocytes in 4 squares} \times 20 \times 10}{4}
\]

Determination of Erythrocyte Sedimentation Rate and Packed Cell Volume

When blood containing anticoagulant is allowed to stand in a perpendicular tube, the erythrocyte sinks because they are heavier than plasma in which they are suspended. ESR was measured easily and accurately.
determined by filling wintrobe hematocrit tube with the help of pasteur pipette. The tube was allowed to stand in a specially constructed rack that held it absolutely perpendicular to the surface of table. This rack was placed in laboratory table which was free from vibration of any sort. The filled tube was allowed to stand for one hour and level of the top of erythrocyte column was recorded. The scale of the wintrobe tube was utilized and the measurement was recorded as the number of millimeter fall per hour.

**Packed cell volume (PCV):**

It was determined after centrifuging the hematocrit tube which was filled for ESR determination for 45 minutes at 3000 rpm. The column of packed RBC were recorded with the scale provided on the tube.

**Differential leukocyte count**

A thin blood smear was prepared after spreading a fresh drop of blood on a well cleaned glass slide. The smear was dried quickly by shaking it in air. The smear was then covered with Leishman's stain and allowed to act for 1 minute. The stain was diluted with double the quantity of distilled water and allowed to remain for ten minutes. Then washed with distilled water, allowed to dry and examined under
oil immersion lense. Two hundred WBC were classified in various types i.e. Neutrophils, lymphocytes, eosinophils, monocytes and their \( t \) was calculated.

v. **Effect of metronidazole infusion on Serum alanine amino transferase (SGPT)**

and **Serum aspartate amino transferase (SGOT) enzyme in Dogs**

Blood samples without anticoagulant were collected for serum collection. To perform these tests non haemolysed serum was required as little haemolysis will give false result. The erythrocyte of the dogs are most fragile and whenever they come in contact with glass surface, get ruptured and give haemolysed sera.

To overcome this difficulty Amrit-Sil-Spray was used. Amrit Sil Spray (Silicon spray in 500 gm. pack) of Batch No.12 dt. 7.2.78 manufactured by Amrut Industrial Products was used. This will form one micron thick layer and ensures penetration into deepest cavity. Prior to use, spray bottle was well shaken. About 15 test tubes of 20 ml. capacity and 15 needles of 18 G. were sterilised in hot air oven at 160\(^\circ\)C for 2 hours. These test tubes and needles were sprayed
with silicon spray. One application will be sufficient even the test tubes were washed several times. Thus the silicon spray prevents the contact of RBC with glass surface and prevents rupture and hemolysis. Test tubes were allowed to dry at room temperature for one hour and rinsed with distilled water to remove excess of silicone spray and was then put again in hot air oven for sterilisation.

At the time of blood collection dry test tubes and needles were used. Blood was collected directly from needle to test tubes. About 5 ml. of blood was collected and allowed it to clot for an hour at room temperature. These test tubes, in which blood was completely clotted, were transferred into the refrigerator. Early in the morning serum samples were collected in another test tube and centrifuged at 3000 rpm for 15 minutes. RBC settled down at bottom giving clear serum above which was then transferred to another test tube by means of Pasteur Pipette.

\[
\text{SGOT} < \text{SGPT}
\]

This test was performed by using standard kit for 50 test (manufactured by M/s. Decruz Corporation, Bombay.) Standard curve for SGOT and SGPT were obtained and from these curves readings were taken of serum samples collected. Readings were taken on Spectronic
20 (Busch and Lomb), at 505 mu wave length. Percentage transmission was calculated by adjusting distilled water blank to 100% transmission. This percentage transmission was then converted to optical density, from which then to the units/ml were calculated by referring standard curves.

Other Tests:

Urine samples were subjected to examination to detect presence of protein. Urine samples were collected before treatment and on 7th day, 14th day and 21st day after treatment. Body weights of each dog on 1st day and 7th day, 14th day and 21st day were recorded.

General Observations:

The animals were observed closely every day to record any toxic symptoms due to metronidazole. Their feed and water intake were also observed. Their general behaviour was observed during the twenty one day of experiment.

Statistical Analysis:

The data was subjected to statistical analysis to test the significance of difference between individuals and between treatments as per Snedecor and Cochran (1967).
OBSERVATIONS AND RESULTS

1. **Pyrogen Test** (Table - X)

Not a single rabbit had shown individual temperature rise above 0.6°C. Calculated sum of response of individual temperature rise for three rabbits was 1.05°C. Maximum limit of summed responses admissible according to Indian Pharmacopoeia (Supplement 1975) is 1.4°C for a group of 3 rabbits. As summed of responses was less than 1.4°C and not a single rabbit had shown rise in temperature above 0.6°C, therefore, material under study was pyrogen free.

11. **Acute Toxicity study in Mice** ... (Table - IX)

No untoward effects were seen at doses 50 mg/kg. and 150 mg/kg. when given by intravenous route. However, at doses 200 mg/kg. and 250 mg/kg., slight transitory CNS depression was observed for one hour, but recovery was rapid. Control group had received only normal saline by intravenous route. No untoward effects were recorded in control groups. No mortality was recorded during 24 hours and subsequently for 6 days in any of the groups.

111. **Effect on Cardiovascular system and Respiratory system of dogs.** (Fig. 1, 2 and 3).
Six non descripts dogs of either sex were used in this experiment. No significant changes as rise or fall in blood pressure were recorded after start of infusion. Responses obtained for vaso-pressor drug such as nor-epinephrine and vaso-depressor drug such as acetylcholine before and after infusion of metronidazole did not show any difference. No change in respiration before, during and after infusion metronidazole was observed.

iv. Effect on Haematological parameters of dogs
(21 day study).

Metronidazole was administered to 6 dogs intravenously daily for 7 days. The dose of 100 ml. (5 mg/ml.) was administered in 20 minutes time to each dog. In order to find out the effect due to this drug on haematological parameters and being individual will have difference susceptibility constituent, a two way classification was carried out to test individual differences as well as effect of drug on parameters considered on prior the day of infusion and on 7th day, 14th day and 21st day from onset of infusion.

1. Effect of metronidazole infusion on Haemoglobin
(gms/100 ml.) of dogs: (Table...I)

Means of Haemoglobin was observed on prior the day of infusion and 7th day, 14th day and 21st day from
onset of infusion. Corresponding means were 11.63 ± 0.25, 10.76 ± 0.89, 10.41 ± 0.79 and 11.20 ± 0.76 gms/100 ml. respectively. In order to test the difference between mean in haemoglobin due to effect of metronidazole infusion, analysis of variance was run and it was observed that between treatment effect was non-significant.

However, it was observed in dog No. 4 that metronidazole had depressant effect on haemoglobin percentage. Reduction from 11.6 gms/100 ml. control to 6.4 gms/100 ml. on 7th day, 6.5 gms/100 ml. on 14th day and 7.4 gms/100 ml. on 21st day.

11. Effect of metronidazole infusion on packed cell volume (mm.) of dogs ........ Table IV.

Means of packed cell volume were observed on prior the day of infusion and on 7th day, 14th day and 21st day of observations from onset of infusion. Corresponding means were 42.58 ± 2.59, 41.08 ± 2.72, 42.50 ± 3.47 and 42.83 ± 3.90 mm. respectively. In order to test the difference between mean in packed cell volume due to effect of metronidazole infusion, analysis of variance was applied and it was observed that between treatment effect was non significant.
However, it was observed that dog No. 4 had shown
decreased in packed cell volume consistently from control
to the 3rd week (36, 30, 27 and 26 \text{ mm}). Also dog
No. 3 had shown fall from 47.00 \text{ mm} control to 36.00 \text{ mm},
on 7th day, 47.00 \text{ mm} on 14th day and again reduced to
41.00 \% on 21st day. However, dog No. 6 showed instead
consistent rise in packed cell volume after treatment with
metronidazole.

\text{(c) Effect of metronidazole infusion on erythrocyte}
\text{sedimentation rate (mm fall/hour) in dogs.}
\text{........ (Table III).}

Means of erythrocyte sedimentation rate was
observed on prior the day of infusion, on 7th, 14th and
21st day of infusion. Corresponding means were 7.83 \pm 4.25,
5.83 \pm 5.24, 6.50 \pm 6.10 and 7.16 \pm 6.77 mm fall/hour
respectively. In order to test difference between mean in
erythrocyte sedimentation rate per hour due to effect of
metronidazole infusion, analysis of variance was run and it
was observed that in between treatment they did not differ
significantly. However, No.2 dog’s erythrocyte sedimentation
rate per hour was increasing consistently, even after the
infusion. But in No.4 and No.5 this rate was in descending
order.

\text{(d) Effect of metronidazole infusion on total}
\text{erythrocyte count (x 10^6 / cu.mm).} \text{... (Table II).}
Means of total erythrocyte count was observed on
prior the day of infusion and on 7th, 14th and 21st day
from onset. Corresponding means were 5.45 ± 0.22,
4.37 ± 0.41, 4.86 ± 0.40 and 4.98 ± 0.42 million/ cu.mm
respectively. In order to test difference between mean
in total erythrocyte count due to effect of metronidazole
infusion, analysis of variance was applied and it was
observed that difference between treatments were non
significant. However, No.4 dog had shown sudden fall in
of total erythrocyte count from 5.84 million/cu.mm
to 2.95, 2.97 and 3.06 million /cu.mm on 7th, 14th and
21st day.

(c) **Effect of metronidazole infusion on total
leukocyte count (× 10^3 / cu.mm) - (Table V).**

Means of total leukocyte count prior the day of
infusion, on 7th, 14th and 21st day from onset of infusion
were calculated. Respective means were 19.250 ± 0.65,
18.725 ± 1.57, 16.133 ± 1.26 and 16.138 ± 0.77 thousands/
cu.mm. In order to test difference between mean in total
leukocyte count due to effect of metronidazole infusion,
analysis of variance was carried out and it was observed
that between treatment effect was significant ( P ≤ 0.05).

In order to test individual means of leukocyte
count mean on prior the day of infusion, on 7th, 14th
and 21st day from onset of infusion, 't' test was carried
out and it was found that control and 7th day observations,
differ significantly from 14th and 21st day observations.
However, control did not differ significantly from 7th day observation and 14th day observation did not differ significantly from 21st day observation.

(f) Effect of metronidazole infusion on differential leukocyte count. (Table VI, VIa and VI b.)

Means of neutrophils and lymphocytes were observed. Means for neutrophils were 69 ± 1.47, 70 ± 1.35, 71 ± 1.51 and 71 ± 1.43 and means for lymphocytes were 26 ± 1.76, 26 ± 1.42, 26 ± 1.18 and 25 ± 1.43 on control, 7th, 14th and 21st day of observations. Analysis of variance was carried out and it was observed that difference between treatments effects was non significant.

V. 1) Effect of metronidazole infusion on serum alanine amino-transferase (SGPT) units/ml in dogs.... Table VII.

Means of SGPT were 10 ± 0.82, 15 ± 1.29, 9.5 ± 0.87 and 8.5 ± 0.29 units/ml on control, 7th, 14th and 21st day from onset of infusion. Analysis of variance was applied and it was observed that between treatment effect was highly significant (P < 0.01). In order to test individuals means of SGPT on prior the day of observation, 7th, 14th and 21st day after onset of infusion,
't' test was applied and it was found that the means of SCPT on 7th day were much higher than those of means of SCPT on 21st day of infusion.

ii. Effect of metronidazole infusion on serum-aspartate-aminotransferase (SGOT) in dogs:

(Table VIII.)

Means of SGOT unit/ml. were observed on prior the day of infusion and on 7th, 14th and 21st day from onset of infusion. Corresponding means were 38.25 ± 1.18, 44.50 ± 2.63, 37.50 ± 3.30 and 35.75 ± 2.66 units/ml. respectively. Analysis of variance was run and it was observed that between treatments differ significantly (P < 0.05). In order to test individual means of SGOT on prior the day of infusion, on 7th, 14th and 21st day after onset of infusion, 't' test was performed and was found out that 7th day observation differ significantly from control, 14th and 21st day observation. Other did not differ significantly.

Other tests.

Tests on urine were performed for the detection of presence of protein on prior the day of infusion, on 7th, 14th and 21st day from onset of infusion and were found out to be negative.

Body weights were recorded and remained more or less constant before and after infusion (Table XI)
General Observations:

Feed and water intake were normal, for all six dogs throughout the experiment. No toxic symptoms were recorded during and after infusion. There were no any observable changes in the consistency of stool or abnormality in urine samples.
Fig. 1. Effect of intravenous metronidazole on anaesthetised dog. Recordings of blood pressure, ECG, pulse and respiration on multi-channel Polyrite.
**Fig. 2.** Effect of intravenous metronidazole on blood pressure, ECG, pulse and respiration of anaesthetised dogs before, during and after infusion (multichannel polyrite recording).
**Fig. 3.** Effect of intravenous metronidazole on blood pressure and respiration of dogs. Responses to NA and ACh before metronidazole and after metronidazole.
### TABLE I.

Effect of Metronidazole Infusion on Haemoglobin (gms/ 100 ml.) of dogs.

<table>
<thead>
<tr>
<th>Sr. No. of Dogs</th>
<th>Control</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>11.0</td>
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<td>11.0</td>
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<td>4</td>
<td>11.6</td>
<td>6.4</td>
<td>6.5</td>
<td>7.4</td>
</tr>
<tr>
<td>5</td>
<td>11.0</td>
<td>11.6</td>
<td>11.0</td>
<td>11.8</td>
</tr>
<tr>
<td>6</td>
<td>11.4</td>
<td>11.6</td>
<td>11.6</td>
<td>12.6</td>
</tr>
</tbody>
</table>

**Mean**

|          | 11.63 | 10.76 | 10.41 | 11.20 |

**SE**

|          | 0.35  | 0.89  | 0.79  | 0.76  |

---

Analysis of Variance of Haemoglobin (gms/100 ml.)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MSEC</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Individuals</td>
<td>5</td>
<td>42.35</td>
<td>8.47</td>
<td>6.32  **</td>
</tr>
<tr>
<td>Between Treatment</td>
<td>3</td>
<td>5.03</td>
<td>1.67</td>
<td>1.25</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>20.04</td>
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</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>67.41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**P < 0.01**
### TABLE II.

Effect of Metronidazole Infusion on total Erythrocyte count of dogs ( x $10^6$ / cu. mm.)

<table>
<thead>
<tr>
<th>Sr. No. of dogs</th>
<th>Control</th>
<th>7th day</th>
<th>14th day</th>
<th>31st day</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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<td>2</td>
<td>4.56</td>
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<td>3</td>
<td>5.07</td>
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<td>4.99</td>
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<td>4</td>
<td>5.34</td>
<td>2.95</td>
<td>2.97</td>
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<td>5</td>
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<td>5.17</td>
<td>5.20</td>
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</tr>
<tr>
<td>6</td>
<td>6.09</td>
<td>5.07</td>
<td>5.64</td>
<td>5.99</td>
</tr>
</tbody>
</table>

Mean 5.45 4.37 4.86 4.98

SE 0.22 0.41 0.40 0.42

---

Analysis of Variance of Erythrocyte ( x $10^6$ / cu. mm)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MSS</th>
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</thead>
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<td>Between Individuals</td>
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<td>11.03</td>
<td>2.21</td>
<td>4.42 *</td>
</tr>
<tr>
<td>Between treatment</td>
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<td>3.6</td>
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<tr>
<td>Error</td>
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<tr>
<td>Total</td>
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<td>21.22</td>
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<td></td>
</tr>
</tbody>
</table>

* - $P \leq 0.05$
TABLE III.
Effect of Metronidazole Infusion on Erythrocyte sedimentation rate (fall in mm/hour)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Control</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
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<td>nil</td>
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<td>22</td>
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<table>
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<tbody>
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<td>4.25</td>
<td>5.24</td>
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</tbody>
</table>

Analysis of variance of Erythrocyte Sedimentation Rate (mm. fall/hour.)

<table>
<thead>
<tr>
<th>Source of variation</th>
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<th>SS</th>
<th>MSS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Individuals</td>
<td>5</td>
<td>3389.33</td>
<td>677.87</td>
<td>21.71 **</td>
</tr>
<tr>
<td>Between Treatment</td>
<td>3</td>
<td>13.34</td>
<td>4.45</td>
<td>0.14</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>468.33</td>
<td>31.22</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>3871.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** P ≤ 0.01
TABLE IV.

Effect of Metronidazole Infusion on Packed Cell Volume (mm) of dogs.

<table>
<thead>
<tr>
<th>Sr. No. of dogs</th>
<th>Control</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49.0</td>
<td>46.5</td>
<td>48.0</td>
<td>52.0</td>
</tr>
<tr>
<td>2</td>
<td>37.5</td>
<td>46.0</td>
<td>38.0</td>
<td>40.0</td>
</tr>
<tr>
<td>3</td>
<td>47.0</td>
<td>36.0</td>
<td>47.0</td>
<td>41.0</td>
</tr>
<tr>
<td>4</td>
<td>36.0</td>
<td>30.0</td>
<td>27.0</td>
<td>26.0</td>
</tr>
<tr>
<td>5</td>
<td>49.0</td>
<td>43.0</td>
<td>48.0</td>
<td>48.0</td>
</tr>
<tr>
<td>6</td>
<td>37.0</td>
<td>45.0</td>
<td>47.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

| Mean            | 42.58   | 41.08   | 42.50    | 42.83    |
| SE              | 2.59    | 2.72    | 3.47     | 3.90     |

Analysis of variance of Packed Cell Volume (mm)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MSS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Individuals</td>
<td>5</td>
<td>930.87</td>
<td>186.17</td>
<td>8.99 **</td>
</tr>
<tr>
<td>Between Treatment</td>
<td>3</td>
<td>11.25</td>
<td>3.75</td>
<td>0.18</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>310.88</td>
<td>20.72</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>12.53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** - $P \leq 0.01$
TABLE V.
Effect of Metronidazole Infusion on Total leukocyte count (x 10^3 /cm. mm.) of dogs.

<table>
<thead>
<tr>
<th>Sr. No. of dogs</th>
<th>Control</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.00</td>
<td>19.60</td>
<td>19.75</td>
<td>15.95</td>
</tr>
<tr>
<td>2</td>
<td>20.25</td>
<td>22.30</td>
<td>19.20</td>
<td>15.00</td>
</tr>
<tr>
<td>3</td>
<td>16.90</td>
<td>15.45</td>
<td>15.00</td>
<td>14.00</td>
</tr>
<tr>
<td>4</td>
<td>20.15</td>
<td>23.25</td>
<td>15.50</td>
<td>18.85</td>
</tr>
<tr>
<td>5</td>
<td>18.00</td>
<td>13.25</td>
<td>11.25</td>
<td>15.03</td>
</tr>
<tr>
<td>6</td>
<td>21.20</td>
<td>18.60</td>
<td>16.10</td>
<td>18.00</td>
</tr>
</tbody>
</table>

| Mean            | 19.250\(^a,b\) | 18.725\(^a,b\) | 16.133   | 16.138   |

| SE              | 0.65   | 1.37   | 1.26    | 0.77     |

Analysis of variance of Leukocyte (x 10^3/cm.mm.)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MSS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Individuals</td>
<td>5</td>
<td>92</td>
<td>18.4</td>
<td>4.54</td>
</tr>
<tr>
<td>Between treatment</td>
<td>3</td>
<td>49.62</td>
<td>16.54</td>
<td>4.08</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>60.76</td>
<td>4.06</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>202.38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P ≤ 0.05
**TABLE VI.**

Effect of Metronidazole infusion on differential leukocyte count in dogs.

<table>
<thead>
<tr>
<th>Sr. No. of dogs</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>(5)</th>
<th>(6)</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>69</td>
<td>66</td>
<td>69</td>
<td>65</td>
<td>71</td>
<td>75</td>
<td>69</td>
<td>1.47</td>
</tr>
<tr>
<td>L</td>
<td>23</td>
<td>29</td>
<td>25</td>
<td>32</td>
<td>24</td>
<td>20</td>
<td>26</td>
<td>1.76</td>
</tr>
<tr>
<td>Control M</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>7th day</td>
<td>N</td>
<td>71</td>
<td>67</td>
<td>70</td>
<td>67</td>
<td>70</td>
<td>76</td>
<td>70</td>
</tr>
<tr>
<td>L</td>
<td>24</td>
<td>28</td>
<td>27</td>
<td>30</td>
<td>26</td>
<td>20</td>
<td>26</td>
<td>1.42</td>
</tr>
<tr>
<td>M</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>14th day</td>
<td>N</td>
<td>67</td>
<td>69</td>
<td>71</td>
<td>69</td>
<td>74</td>
<td>77</td>
<td>71</td>
</tr>
<tr>
<td>L</td>
<td>23</td>
<td>29</td>
<td>26</td>
<td>25</td>
<td>24</td>
<td>21</td>
<td>26</td>
<td>1.18</td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>21st day</td>
<td>N</td>
<td>72</td>
<td>66</td>
<td>67</td>
<td>74</td>
<td>73</td>
<td>75</td>
<td>71</td>
</tr>
<tr>
<td>L</td>
<td>23</td>
<td>29</td>
<td>30</td>
<td>23</td>
<td>24</td>
<td>21</td>
<td>25</td>
<td>1.48</td>
</tr>
<tr>
<td>M</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

N - Neutrophil
L - Lymphocyte
M - Monocyte
E - Eosinophil
### TABLE VI.a.

**Analysis of variance of Neutrophils**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MSS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Individuals</td>
<td>5</td>
<td>205.5</td>
<td>41.11</td>
<td>13.89 **</td>
</tr>
<tr>
<td>Between Treatment</td>
<td>3</td>
<td>12.51</td>
<td>4.17</td>
<td>1.41</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>44.49</td>
<td>2.96</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>23</td>
<td>262.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** *P ≤ 0.01**

### TABLE VI.b.

**Analysis of variance of Lymphocyte**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MSS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Individuals</td>
<td>5</td>
<td>175.21</td>
<td>35.04</td>
<td>6.06 **</td>
</tr>
<tr>
<td>Between Treatment</td>
<td>3</td>
<td>2.13</td>
<td>0.71</td>
<td>0.12</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>86.63</td>
<td>5.78</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>23</td>
<td>263.96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** *P ≤ 0.01**
TABLE VII.

Effect of metronidazole infusion on Serum-alanine- 
amino-transferase test ( SGPT ) in dogs. ( Unit /ml.)

<table>
<thead>
<tr>
<th>Sr. No. of dogs</th>
<th>Control</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>10</td>
<td>14</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>16</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>18</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>12</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Mean</td>
<td>10</td>
<td>15</td>
<td>9.5</td>
<td>8.5</td>
</tr>
<tr>
<td>SE</td>
<td>0.82</td>
<td>1.29</td>
<td>0.87</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Analysis of variance of serum-alanine-amino-transferase ( SGPT )

<table>
<thead>
<tr>
<th>Source of variations</th>
<th>DF</th>
<th>SS</th>
<th>MSS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Individuals</td>
<td>3</td>
<td>5.5</td>
<td>1.83</td>
<td>0.51</td>
</tr>
<tr>
<td>Between treatment</td>
<td>3</td>
<td>101.0</td>
<td>33.67</td>
<td>9.33**</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>32.5</td>
<td>3.61</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>139.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** - P ≤ 0.01
TABLE VIII.
Effect of Metronidazole Infusion on serum-aspartate-aminotransferase (SGOT) test in dogs (Unit/ml.)

<table>
<thead>
<tr>
<th>Sr. No. of dogs</th>
<th>Control</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>40</td>
<td>52</td>
<td>42</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>44</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>42</td>
<td>44</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>40</td>
<td>34</td>
<td>34</td>
</tr>
</tbody>
</table>

Mean 33.25 44.50 37.50 35.75
SE 1.18 2.63 3.30 2.66

Analysis of variance of Serum-aspartate-aminotransferase (SGOT)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MSS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Individuals</td>
<td>3</td>
<td>181.5</td>
<td>60.5</td>
<td>4.06 *</td>
</tr>
<tr>
<td>Between Treatment</td>
<td>3</td>
<td>174.5</td>
<td>58.17</td>
<td>3.91 *</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>134.0</td>
<td>14.89</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>490.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* - p ≤ 0.05
TABLE IX.

Acute Toxicity study of Metronidazole by Intravenous Route in Mice.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Dose</th>
<th>Observations</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50 mg/kg</td>
<td>No untoward effect</td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>100 mg/kg</td>
<td>No untoward effect</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>150 mg/kg</td>
<td>No untoward effect</td>
<td>Nil</td>
</tr>
<tr>
<td>4</td>
<td>200 mg/kg</td>
<td>Slight transitory CNS depression for one hour.</td>
<td>Nil</td>
</tr>
<tr>
<td>5</td>
<td>250 mg/kg</td>
<td>Slight transitory CNS depression for one hour.</td>
<td>Nil</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>No untoward effect</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>(N.S)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**TABLE X.**

Pyrogen test for metronidazole (metyrogyl) injection in rabbits.

**Temperature Chart:**

Degree of Centigrade (°C)

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Before Administration of drug (Minutes)</th>
<th>After Administration of drug (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0  30  60  90  Average  30  60  90  120  150  180</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>38.8 38.7 38.7 39.00 38.80 38.7 38.6 38.7 39.1 39.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>38.7 38.7 38.6 38.9 38.70 39.2 39.0 39.2 39.2 39.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>38.8 39.1 38.9 39.0 38.95 38.8 38.8 39.0 38.9 38.9 39.2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Maximum Temp.</th>
<th>Mean Temp.</th>
<th>Response (Difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39.10</td>
<td>38.80</td>
<td>0.30</td>
</tr>
<tr>
<td>2</td>
<td>39.20</td>
<td>38.70</td>
<td>0.50</td>
</tr>
<tr>
<td>3</td>
<td>39.20</td>
<td>38.95</td>
<td>0.25</td>
</tr>
</tbody>
</table>

**Summed Response**

... 1.05

**Individual Rabbit Showing**

Temperature rise above 0.6°C. ... Nil

**Maximum limit of summed response admissible according to I.P. (supplement 1975) is 1.4°C for group of 3 rabbits.**

**Remarks:** Material is pyrogen free.
**TABLE XI**

Effect of Metronidazole Infusion on body weight of dogs (kg).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Control</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.5</td>
<td>12.5</td>
<td>13.0</td>
<td>12.5</td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
<td>12.5</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>3</td>
<td>15.0</td>
<td>15.0</td>
<td>14.5</td>
<td>15.0</td>
</tr>
<tr>
<td>4</td>
<td>16.0</td>
<td>16.0</td>
<td>16.0</td>
<td>16.0</td>
</tr>
<tr>
<td>5</td>
<td>13.0</td>
<td>12.5</td>
<td>13.0</td>
<td>13.0</td>
</tr>
<tr>
<td>6</td>
<td>14.5</td>
<td>14.0</td>
<td>14.0</td>
<td>14.5</td>
</tr>
</tbody>
</table>
DISCUSSION

Metronidazole being sparingly soluble in water, formulating a suitable parenteral preparation was a problem. However, an injectable metronidazole preparation (Metrogyl) was made available for intravenous use by M/s. Unique Pharmaceutical Labs., Bombay. There are two formulations, one containing 5 mg/ml. metronidazole supplied in 100 ml. quantities and 2 mg/ml. metronidazole I.P. and 5% Dextrose I.P. w/v supplied in 350 ml. quantities. These preparations are packed in infusion bottles.

Pyrogen Test in Rabbits.

The results of pyrogen test in rabbits are presented in Table X. The test was performed as per Indian Pharmacopoeia (1955; supplement, 1975 to Pharmacopoeia of India, 1968). Accordingly metronidazole preparation proved to be pyrogen free. This test was conducted because the preparation is intended for intravenous administration.

Acute Toxicity in Mice

The metronidazole was administered intravenously in mice. The results are presented in Table IX. It was observed that the metronidazole when administered to mice upto 150 mg/kg proved to be free from any toxic effects.
However, further doses of 200 mg and 250 mg/kg, produced slight transient CNS depression which lasted for an hour. The study in mice indicated the broad margin of safety and very low toxicity. It was not possible to calculate \( LD_{50} \) value in mice as there was no mortality up to a dose rate of 250 mg/kg. Higher concentrations of above 5 mg/ml were not available as well as total volume of intravenous injection in mice could not be increased to more than 1 ml.

There is very little information in literature regarding toxicity of intravenous metronidazole. In human beings Lewis and Kenna (1969) reported that an attempted suicide by ingestion of 12 gms. of metronidazole resulted into minimal disturbances. Bock (1977) reported that intravenous administration of metronidazole in rats with dosage range of 60, 150 and 300 mg/kg/day for 4 weeks did not produce any toxic symptoms during and after infusion.

**Effect of metronidazole infusion on Respiratory and Cardiovascular system of dogs:**

Effect of metronidazole infusion in dog, especially on blood pressure, heart rate and respiration was studied in 6 anaethetised dogs. It could be appreciated from figs. 1, 2 and 3 that metronidazole injection did not affect the blood pressure and respiration. These parameters were -
recorded on the four channel Polyrite (INCO, Ambala) simultaneously. Kymograph was also used for recording blood pressure and respiration.

Responses for vaso-pressor drug such as nor-epinephrine and vaso-depressor drug such as acetylcholine remained unaffected even after infusion of metronidazole. (Fig. 3.). These observations suggested that there was no blocking effect on the responses of these vaso-active drugs. The intravenously administered metronidazole at the dose levels 50 to 70 mg/kg, did not affect the normal pattern of blood pressure and respiration. Eovell (1967) and Bost (1977) had similarly observed in their independent studies, that metronidazole had no effect on blood pressure and respiration in experimental animals. The present study thus is in agreement with these workers and it clearly indicated the safety of the intravenous metronidazole preparation. The doses studied in animals were 4 to 5 times higher than the expected therapeutic dose in human beings.

**Effect of Metronidazole infusion on Haematology of Dogs - (21 day study)**

Injectable metronidazole 100 ml. (5 mg/ml.) was administered to each of six dogs daily for 7 days, intravenously. The initial haematology was performed as
control reading before the administration of metronidazole. The total dose of 100 ml. was administered by slow intravenous injection over a period of 20 minutes.

**Effect on Haemoglobin**

The results of effect of metronidazole on haemoglobin are presented in Table I. All the dogs showed basal haemoglobin values within normal limits. However, one dog (dog No. 4) out of six dogs showed a substantial decrease in haemoglobin values, at first and second weeks. It was not clear whether this haemoglobin depression in a group of six could be counted as individual variation. This particular study (Haemoglobin) should be performed in the larger group of population to clarify the doubt.

**Effect of metronidazole infusion on packed cell volume (PCV).**

The results are presented in Table IV. Two dogs (No. 3 and No. 4) in the group had shown fall in PCV values. However, a tendency for recovery was observed at the end of 3rd week. Surprisingly one dog (No. 6) showed consistent increase in PCV. The results obtained were not significant for the group, but were significant between individuals.
Irrespective of increased or decreased PCV values observed in a few dogs, there was a tendency of recovery at the end of 21st day. Therefore, the effects of metronidazole on PCV if any, appeared to be transitory.

**Effect of Metronidazole Infusion on Total erythrocyte count.**

The results were presented in Table II. All the animals except one (No.4) showed very little changes in erythrocyte count after treatment. Dog No.4 showed decrease in erythrocyte count. Statistically no significant results were obtained in between treatment.

The haematological picture for the dog No.4 is same as far as haemoglobin, packed cell volume and erythrocyte count are concerned. In order to understand whether this difference in dog No.4 was due to any internal blood loss, the animal was sacrificed at the end of 3rd week and post-mortem was performed. On gross observations of the vital organs, it was found that there was no indication of internal haemorrhage. The mucus membrane was found pale. The observations in dog No.4 regarding the depression of haemoglobin and other parameters suggest that further studies in larger size of population are necessary. Brodgen et al. (1973) reported that chemicals structurally related to
metronidazole caused blood dyscrasias, but serious difficulties have not been recorded with metronidazole. 

Even though the haematological differences in Dog No. 4 appeared to be due to individual variation, it warrants further studies in a larger group of animals.

**Effect of Metronidazole infusion on erythrocyte sedimentation rate.**

The results are presented in Table III. Dog No. 2 showed consistent rise in erythrocyte sedimentation rate. The results were not statistically significant between treatment. However, individual rise in erythrocyte sedimentation rate may be indicative of inflammatory changes.

**Effect of Metronidazole infusion on total leukocyte count**

It has been observed from Table V that metronidazole had affected the total leukocyte count in dogs. There was significant decrease in total leukocyte count after 1st, 2nd and 3rd weeks. The difference was significant on 14th and 21st day. Metronidazole has shown significant suppression of leukocyte count in individuals and between treatments. However, Bost (1965) had found no change in haematological values after intravenous administration
for 4 weeks. Lefebvre and Hesseltine (1965) had reported neutropenia in individuals at therapeutic doses. But these changes were reversible after withdrawal of treatment.

**Effect of Metronidazole infusion on differential leukocyte count**

Results were presented in Table VI, VIa and VIb. No appreciable changes were seen in differential leukocyte count was observed after intravenous metronidazole infusion.

**Effect of Metronidazole infusion on Liver Function Tests - serum-alanine- amino-transferase (SGPT) and serum-aspartate- amino-transferase (SGOT)**

It has been observed that SGPT values differed significantly after treatment. (Table VII). The study was performed on four dogs. At the end of 1st week there was significant rise in SGPT values and at the end of 2nd and 3rd weeks the values showed tendency to recover. This indicated that SGPT values were affected by metronidazole eventhough for a short period.
Similarly SGOT values (Table VIII) differed significantly after treatment and this effect was also observed at the end of 1st week. Tendency for recovery was seen in three dogs but one dog (No.4) did not show any recovery and the values for SGOT were decreased at the end of 2nd and 3rd week. Bost (1977) had shown that sub-acute and chronic studies in the monkey with oral metronidazole (45 to 240 mg/kg/day) revealed a drug-related effect on liver as evidenced by histological examination, without associated changes in serum enzyme levels. However, under the present study doses between 30-50 mg/kg for 7 days had produced transitory increase in serum enzyme levels which returned to normal by 2nd and 3rd weeks.

Miscellaneous Tests

Tests performed for the detection of presence of protein in urine were found to be negative, indicating that metronidazole was not harmful to renal function. Hykyn and Phillips (1976) had reported that reduction in dosages of intravenous metronidazole was not warranted in renal failure cases.

Body weights of the dogs did not show any appreciable changes during experiments for 3 weeks. Bost
(1977) also reported no influence of metronidazole on four week studies on body weights.

There were no observable toxic symptoms during a period of 3 weeks. Bost (1977) had found marked CNS effect in dog at doses 150-225 mg/kg. metronidazole when administered intravenously.

From above discussion it is quite evident that metronidazole can be used safely by intravenous route. However, further studies on haematological parameters in a larger group of population of dogs and also other species are warranted.
SUMMARY

Metronidazole is used in various clinical conditions viz. amoebiasis, anaerobic infections etc. There is very limited information in the literature regarding the parenteral preparation of the metronidazole. The intravenous preparation of the metronidazole obviously will have certain additional advantages from bioavailability point of view.

Some toxicity studies were conducted on the metronidazole preparation meant for intravenous use. The metronidazole preparation (Metrogyl) was formulated by M/s Unique Pharmaceutical Labs., Bombay and was supplied for this project. The concentrations supplied were 5 mg/ml. and 2 mg/ml. in 5% Dextrose w/v packed in 100 ml. and 350 ml. infusion bottles respectively.

The following parameters were studied in the present project on metronidazole infusion -

I. Pyrogen Test in Rabbits.

II. Acute Toxicity study in mice.

III. Effect on cardiovascular and respiratory system of anaesthetised dog.

IV. Effect on haematological parameters (21 day study) in dogs.

i. Haemoglobin.
ii. Packed cell volume.

iii. Erythrocyte sedimentation rate.

iv. Total erythrocyte count.

v. Total leukocyte count.

vi. Differential leukocyte count.

V. **Effect on liver function test:**

1. Serum-alanine-amino-transferase (SGPT)

2. Serum-aspartate-amino-transferase (SGOT)

VI. **Miscellaneous Test**

Effect on body weight, urine examination for detection of protein.

VII. **General Observations**

Toxic symptoms, behaviour of animals and food and water intake.

The metronidazole preparation was found to be free from any pyrogen when tested in rabbits. There was no mortality in mice when metronidazole was administered intravenously up to 250 mg/kg. However, there was transient depression noted in case of mice receiving metronidazole at the dosages of 200 mg/kg and 250 mg/kg.

There were no observable effects on the basal blood pressure and respiration of the anaesthetised dogs when
metronidazole 50 mg/kg and 70 mg/kg were administered for a period of 20 minutes and 45 minutes respectively. The recording of the blood pressure, respiration, ECG and pulse of dog on multichannel Polyrite also did not indicate any effect of metronidazole infusion.

There were significant changes in total leukocyte count after the metronidazole infusion for 7 days in case of dogs. Total leukocyte counts were suppressed till the end of 3rd week. This result was statistically significant, indicating that total leukocyte count falls down after infusion of metronidazole for 7 days. The changes in respect of other haematological parameters were not statistically significant, however, individual variations were observed.

Liver function tests, serum-alanine-aminotransferase (SGPT) and serum-aspartate-aminotransferase (SGOT), showed significant increase at the end of first week, but reached the normal values at 2nd and 3rd week. There were no any other toxic symptoms exhibited by the experimental dogs during the period of 3 weeks study.

The present study on intravenous metronidazole indicated that the drug possesses high margin of safety with some side effects.
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