Tropical environment and disease

The importance of geographical and environmental factors in determining the prevalence, incidence, progress, recovery and relapse of tropical diseases is well recognised. However, a multi-disciplinary approach is required to tackle the health problems and lessen the burden of diseases in over-populated, over-crowded, poorly developed and economically backward countries. The measurement of health needs in the tropics itself is beset with problems. Although sufficient knowledge exists with regard to the nature of the problem, quantitative data with respect to the extent are not always available. Health problems and diseases in the tropics are closely linked with other factors like the social and economic structure, culture and religion, food production and distribution, and other factors like lack of transportation facilities (communication), unhygienic surroundings, illiteracy and over-population. Health resources in these underprivileged countries are hardly comparable with other well developed countries and are certainly inadequate with respect to the health needs of the population.
In countries like China, Japan, Srilanka and India institutional forms of professional education and practice have been adapted to indigenous medical traditions. However, with advances in physiology, chemistry, biochemistry and other related disciplines and infiltration of modern medicine (western) into the tropics, the modern age of pharmacology has come into existence. Although the flow of drugs has been large and has greatly contributed towards improved health standards, due to certain other closely linked detrimental factors, tropical diseases and the problems associated with them still continue to pose an increasing threat to the welfare of mankind (K.Krishnaswamy, 1979).

One of the most important practical measures in the management of disease is the practice of modern medicine.

Clinical pharmacological considerations

(a) Determinants of dose and dosage schedules in a tropical environment

Drug therapy in tropical countries has a profound influence on health statistics. However, rational use of drugs based on pharmacokinetic and pharmacodynamic studies in these populations is lacking for obvious reasons. Drug dosage as recommended in textbooks and as advised by the
pharmaceutical manufacturer are followed by clinicians. Most of these dosage regimens are standardized on a western population who are genetically different and are also placed in a different environment. Populations differ widely in relation to their physiological and/or biochemical constitution. The genetic profile, body weight, nutritional status and the environment are four major factors which have a direct bearing on drug dosage.

a-1 Genetic profile

The literature is replete with evidence that genetic factors profoundly influence drug metabolism, efficacy and toxicity of xenobiotics (Vessell, 1974).

a-2 Body weight

Most of the studies on drugs take into consideration body weight and doses are often fixed for an average weight of 70 kg. These doses are then extrapolated to other populations and it is a usual practice to follow absolute doses without relevance to body weight. However, on an average, the weight of an adult in developing countries is about 55 kg and consequently most Asians, for example, receive about 20 to 30% more drug/dose based on recommendations for most developed populations (Salafsky, 1976). This problem may be more acute in children since dosage
of a number of drugs is based on age, and for the same age, the body weights and growth patterns are entirely different for the well developed western and underdeveloped eastern populations (Teoh, 1977). Thus, drug doses should be recommended on a body weight basis, rather than on the basis of age or an absolute fixed quantity.

3 Nutritional status

Of all the environmental factors which may influence drug response, nutrition may be one of the most important. The nutritional status of an individual can considerably modify a number of pharmacokinetic processes (Krishnaswamy, 1978), which may ultimately determine the efficacy and side effects of drugs.

The problem of malnutrition in tropical and subtropical countries is enormous. It is usually complex; many deficiencies occurring simultaneously. Changes occur in almost every organ of the body (Krishnaswamy, 1978). Apart from protein energy malnutrition; vitamin, mineral and trace deficiencies will further modify the pathological changes and metabolic and functional responses. Nutritional-pharmacological interactions will determine drug responses and therefore these have to be evaluated carefully. Drug dosages may have to be altered depending on the
nutritional status, the type of nutrient deficiency and the extent of change in the pharmacokinetics of a drug.

Environment

Some factors which determine the metabolism of and response to drugs are related to the physical and chemical environment (Sanvordeker and Lambert, 1972). Higher altitudes have been shown to alter drug responses. For example, impairment in performance due to the antihistamine chlorpheniramine, increases as a function of increasing altitude (Higgins et al., 1968). Light has also been shown to modify metabolism and response to drugs (Nair and Casper, 1969). Relative humidity in an environment can also alter phototoxic responses to topically applied drugs (Levine and Harper, 1969). Change in ambient temperature has a pronounced effect on toxicity of drugs; griseofulvin levels in the skin are higher in summer as compared with winter months (Epstein et al., 1972). Therefore, it seems likely that in extreme weather conditions, such as those experienced in tropical and subtropical areas, drug distribution, phototoxicity and metabolic disposition may be altered by climatological factors. Epidemiological factors like differences in disease pattern, intestinal microflora, atmospheric contamination and mycotoxins may further modify drug response. Systematic studies of drug response in
relation to the physical and chemical environment, particularly in tropical countries, have yet to be undertaken.

(b) Drug pharmacokinetic processes as influence by a tropical environment

It is a well recognized fact that drug pharmacokinetics can be influenced by a number of internal and external variables. Apart from intrinsic factors like age, sex and species, environmental factors like temperature, altitude, light exposure, atmospheric pollution and other climatological factors play an important role in determining many biological processes. In a tropical environment, there are a number of factors such as malnutrition, infectious diseases, parasitic infections, naturally occurring toxins and contaminants and food habits, which have a profound influence on the way in which a drug is handled by individuals (Krishnaswamy, 1978). Very little information is available on this aspect in tropical countries and it appears to be an area open for research activity.

The environment around a subject or a patient prior to, during and after drug administration may have an important influence on drug response in terms of both clinical efficacy and adverse drug reactions. It is possible that extremes of environmental temperature and other
co-existent epidemiological and socio-cultural factors may alter significantly some of the important pharmaco-kinetic processes of drug absorption, distribution, protein binding, biotransformation and excretion.

b-1 Drug absorption and bioavailability

The extent of absorption of an orally administered drug is modified by a number of factors like the pH in the intestinal lumen, gastric emptying rate, intestinal transit time, surface area of the gastrointestinal tract and mesenteric blood flow. In addition, drug metabolism by gut bacteria and by enzymes in the gut wall and presence of infections and infestations may influence the amount of active drug passing across the mucous membrane. In tropical countries the effect of malnutrition and malabsorption syndromes coupled with worm infestation and bacterial infec-tion, can modify the extent of absorption and availability of orally administered drugs. The gastrointestinal tract, both histologically and functionally, is said to be different in a tropical environment (Rosenberg and Scrimshow, 1972). These alterations have been used to explain malabsorption of nutrients. However, in a tropical environment, there are very few investigations on drug absorption in general and of the effect of malnutrition in particular.
Absorption studies need to be undertaken, particularly in the presence of parasitic and bacterial infections.

In addition, different diets per se may modify drug absorption. Populations in different parts of the world and individuals within the same population differ widely in their diets. Therefore, it is not possible to extrapolate data from one country to another. Hence, apart from nutritional status, food habits may profoundly influence drug absorption and the steady-state concentration of drug.

b-2 Drug protein binding and distribution

Drug protein binding has many clinical implications. Such interactions frequently determine the rate at which drugs are absorbed from the gastrointestinal tract, transported to tissues and eliminated from the body. Drug protein interactions are usually reversible, with the free drug in plasma being in equilibrium with that in tissue fluids. Protein energy malnutrition alters plasma and tissue proteins quantitatively and probably also qualitatively. Association (binding) of drugs with proteins, particularly albumin and tissue proteins, is of importance in drug distribution and influences drug delivery to receptor sites of action and to metabolic sites of elimination. Albumin is the main binding protein in plasma and hypoalbuminaemia is a characteristic finding in malnutrition.
The rate of albumin synthesis is directly related to the level of protein intake and amino acid supply (Waterlow, 1975). The profile of plasma proteins (albumin, globulins and other carrier proteins) is altered in protein energy malnutrition. Glycoproteins, particularly al-acid glycoproteins which transport basic drugs, are also likely to be altered in malnutrition. The relationship of concentration of free drug to total drug may vary as a function of drug concentration, total protein concentration and albumin concentration. However, other physicochemical and pharmacokinetic properties also influence disposition of the drug and the magnitude of its pharmacological activity. Almost every drug exerts its pharmacological effect by interacting with some kind of protein in the body.

Since infectious diseases are very common in tropical countries and a number of antibiotics are commonly employed in their treatment, it is important to discuss the clinical relevance of antimicrobial therapy in relation to plasma protein binding. The effect of protein binding on the *in vitro* activity of antimicrobial agents in serum has been extensively studied (Craig and Kunin, 1976; Craig and Welling, 1977). It is reasonably clear from many observations that only the free drug in serum is available for antimicrobial action. Therefore, changes in binding of antimicrobial agents to serum proteins would be expected
to influence the therapeutic effect \textit{in vivo}. It is difficult to generalise on the influence of changes in binding on drug disposition and response. It will be determined by a number of other factors like the physico-chemical properties of the drug, ability of the drug to bind to tissue proteins and cellular membranes which will determine cellular transport, elimination and toxicity, and importantly on inter-related kinetic process of clearance.

Buchanan (1977) has indicated that protein binding \textit{in vitro} of a number of drugs such as salicylate, warfarin, digoxin and thiopentone is altered in malnourished children. However, the clinical implications, particularly in actual clinical use situations, have yet to be evaluated in most cases.

\textbf{b-3 Drug biotransformation}

Drugs and other compounds which are foreign to the body (xenobiotics) have to be eliminated from the body. Most drugs are metabolized in the liver by metabolizing enzymes present in the microsomal, mitochondrial and soluble fractions. Therapeutic efficacy and toxicity of such drugs are directly related to their rate of metabolism by the liver.
The implications of liver disease in relation to drug therapy are obvious and need not be elaborated here. However, it is important to discuss the common liver diseases or conditions with liver involvement which are encountered in tropical countries. These may be broadly divided into four groups: (a) malnutrition; (b) parasitic infestation; (c) liver injury due to mycotoxins; and (d) cirrhosis and/or malignancy. Malnutrition is a major public health problem in tropical countries and fatty infiltration with impaired synthesis of lipoproteins is a characteristic feature in severe protein calorie malnutrition (Krishnaswamy, 1978). More commonly, however, one encounters only vacuolation in the liver. Involvement of the endoplasmic reticulum is also one of the features in protein energy malnutrition (Bhamarapravati, 1975). Therefore, it is important to evaluate whether such changes impair the mixed function oxidases involved in drug metabolism. The literature is replete with evidence that nutritional constraints in experimental animals impair drug metabolizing enzymes (Campbell, 1977). However, studies in experimental animals cannot be directly extrapolated to man because of large species variation in drug metabolizing enzymes. Nevertheless, studies in human subjects (Krishnaswamy and Naidu, 1977) with varying grades of malnutrition do indicate that
the mixed function oxidase system may be affected in severe malnutrition. Vitamin C and iron deficiency have been shown to result in altered antipyrine kinetics in human subjects iron deficiency anaemia resulting in accelerated metabolism and vitamin C deficiency resulting in a decreased rate of metabolism (Langman and Smithard, 1977). However, administration of vitamin C which is sulphated in the body, has been shown to prolong the half-life of paracetamol in man by competing for available sulphate (Houston and Levy, 1976).

Nutritional-pharmacological interaction is a relatively young field of science and only very recently have nutritionists become aware of such interactions (Hatchcock and Coon, 1978). The long term sequelae, particularly the possible development of cirrhosis consequent to severe malnutrition, has long been debated. The relationship between malnutrition and liver diseases has been overemphasized (Rubin and Lieber, 1968). Several studies show that there is in fact no cause and effect relationship (Ramalingaswami and Nayak, 1970; Keet et al., 1971). However, cirrhosis of the liver is commonly encountered in adults in the tropics. In addition, Indian childhood cirrhosis and idiopathic portal hypertension predominate in certain areas. Naturally occurring hepatotoxins aflatoxins as well as viral hepatitis and probably a host
of other unknown factors may be aetiologically related. Liver function, particularly in relation to drug biotransformation in such situations may be impaired. Hepatic blood flow and hepatic extraction of drugs may also be different in such conditions.

It is obvious that in tropical countries, a number of factors can modify the mixed function oxidase drug metabolizing enzyme system and therefore drug response. Only future studies can clarify the nature of and the mechanisms involved and establish a basis for rational use of drugs.

b-4 Excretion of drugs

The two most important organs involved in the excretion of foreign compounds are the liver and the kidney. As mentioned in section b-3, liver involvement can modify hepatobiliary excretion and enterohepatic circulation of drugs, particularly in cases with obstructive pathology. Similarly, tropical diseases can result in kidney involvement (renal lithiasis) and have the potential to interfere with glomerular filtration, tubular secretion and reabsorption of drugs eliminated by renal mechanisms depending on the protein binding of the drug, renal plasma flow, urinary pH and the extent of tubular involvement (reabsorption).
b-5 Consequences of altered pharmacokinetics

Changes in pharmacokinetics of drugs will ultimately be reflected in altered blood concentrations and clinical response. Associated with these, the incidence of adverse reactions and drug interaction with some drugs and susceptibility to organ damage due to genetic variation and nutritional influence for example, may all the expected to be different in a tropical environment.

Consequences of altered pharmacokinetics in a tropical environment are -

1. altered drug plasma concentration
2. alteration in clinical response
3. Changes in incidence of toxic and adverse drug reactions, including drug interactions, and
4. increased susceptibility to organ damage.

Drug response in a tropical environment will ultimately be the net result of a number of influencing factors including malnutrition and the type and degree of...
Malnutrition, co-existing disorders; the genetic constitution and age of the patient; the environment in terms of exposure to chemicals and other pollutants; and the physico-chemical properties and pharmacokinetic characteristics of the particular drug. The extent of change in pharmacokinetics will certainly determine the drug dosage regimen. Tissue response to the drug may also be altered in a tropical environment.

Factors determining drug response (therapeutic and toxic) in a tropical environment are as illustrated below:

| Malnutrition and other diseases or traits peculiar to tropical countries | Environment |
| 1. Age of onset | Drug and Metabolite |
| 2. Severity of the disease | 1. Pollution (chemical) |
| 4. Genetic variation | Response |
| | 3. Smoking and alcoholism |

Drug

1. Physico-chemical and pharmacokinetic properties of the drug
2. Extent of change in pharmacokinetics
3. Indication for the drug
4. Dosage of drug given
(c) Altered tissue responsiveness to drugs as influenced by a tropical environment

One of the most difficult areas of study is clinical pharmacology is to quantitate the effect of drugs in relation to plasma concentrations so as to indicate tissue uptake and response to the therapeutic agent. Apart from clinical response, there are very few pharmacokinetic studies in human subjects. Clinical response is determined by a number of factors and hence objective methods of evaluation have to be developed for each drug before one can talk about tissue response. However, a few studies indicate that disposition of drugs can be altered by factors such as infection and pyrexia.

In acute falciparum malaria, the hepatic metabolism of quinine is impaired leading to higher plasma concentrations and more frequent side effects, probably as consequences of higher tissue uptake of the drug. Influenza and adenovirus infection have been shown to prolong theophylline half-life during the acute stage of the infection and increase the risk of toxicity (Chang et al., 1978). In undernutrition, tissue uptake of tetracycline has been shown to be poor (Shastri and Krishnaswamy, 1976). In febrile conditions, due to cardiovascular changes associated with fever, gentamicin blood levels are
lower due to a shift of the drug from plasma to other body fluid compartments (Pennigton et al., 1975), which may result in therapeutic failure. The hypotensive response to propranolol seems to be less in Kenyan subjects, particularly in the presence of severe undernutrition (Obel and Vere, 1978). Similarly, some Negro patients require a higher dose of prazosin to control blood pressure than Caucasian hypertensives (Falase et al., 1976; Mroczek et al., 1974), probably indicating a higher tissue requirement. Such isolated studies indicate an altered tissue response which may not necessarily be accompanied by changes in kinetics.

(d) **Protein calorie deficiency in tropical environment**

Protein calorie or protein energy malnutrition (PCM, PEM) is one of the most important and widespread nutritional disorders, accounting for high child mortality and morbidity (Gopalan and Srikantia, 1973; Olson, 1977). It includes many different clinical syndromes all of which are accompanied by retardation of growth and development, with protein manifestations. As the name implies, it is due to inadequate food intake. Both calories and proteins are inadequate and chronic dietary inadequacy over a period of time results in the extreme forms of malnutrition in children - kwashiorkor and marasmus. The interplay
between malnutrition and infection (acute, respiratory and gastrointestinal; chronic like pulmonary tuberculosis) is of considerable importance in developing countries; one leading to the other through a vicious cycle.

Tuberculosis

Epidemiology

Tuberculosis is a specific communicable disease caused by M. tuberculosis. It affects both the pulmonary and extrapulmonary tissues.

It may be acute or chronic (generalized or localized).

It is existing in India for more than 2000 years. The disease was called 'pthysis' which means to dry up.

Extent of problem

Tuberculosis is a public health problem of major importance in almost all developing countries. There is no single country which has succeeded in reaching the patient control i.e. less than 1% tuberculin positivity among children in the age group 0-14 years.

Death rates have declined in developed countries but in less developed countries, the problem of tuberculosis continues to be acute with death rates between 60-80/100,000/year.
The number of infectious cases of tuberculosis in the world today is 15-20 million. This infectious pool is maintained by the occurrence of 1-2 million new cases and 1-2 million deaths in the world.

Incidence and prevalence

1. Incidence of infection  1%
2. Incidence of disease  1/1000
3. Prevalence of disease  4/1000
4. Prevalence of infection  30%

<table>
<thead>
<tr>
<th>Age group</th>
<th>Infected %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4 years</td>
<td>1.0</td>
</tr>
<tr>
<td>5-9 years</td>
<td>6.4</td>
</tr>
<tr>
<td>10-14 years</td>
<td>15.4</td>
</tr>
</tbody>
</table>

5. Rural and urban areas have the same prevalence.

6. Fate of patients
50% die within 5 years, maximum deaths occurring in the first year, 30% get well and 20% remain infective.

7. Mortality
60-80/100,000/year

Life span of tuberculous patients has increased due to more effective chemotherapy available.

Adults are a source of infection to children.
<table>
<thead>
<tr>
<th>Types of TB</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrathoracic</td>
<td>41.0</td>
</tr>
<tr>
<td>Tuberculous Meningitis (TBM)</td>
<td>27.5</td>
</tr>
<tr>
<td>Bones and joints</td>
<td>8.9</td>
</tr>
<tr>
<td>Lymph Nodes (superficial)</td>
<td>7.4</td>
</tr>
<tr>
<td>Abdominal</td>
<td>6.9</td>
</tr>
<tr>
<td>Acute miliary</td>
<td>3.9</td>
</tr>
<tr>
<td>Isolated foci</td>
<td>3.9</td>
</tr>
<tr>
<td>Skin</td>
<td>0.2</td>
</tr>
</tbody>
</table>

It is evident from these data that intrathoracic tuberculosis is most common accounting for 41% of cases and pulmonary tuberculosis is a variety of this group.

Tuberculosis, one of the major public health problems in the developing countries of the world today, has made its impact felt throughout the ages. No other disease has so much sociological, economic and health significance as tuberculosis has. From an overview of the prevalence of the disease, it appears that it is an index of the stage of social organization and standards of living of the community.

**Tuberculosis in ancient times**

From the various skulls and other bones which have been recovered from different parts of the world, tuberculosis was found to be evident in Neolithic man. Images
of hunchbacks had been left by ancient civilizations. The Egyptians of antiquity made statuettes, engravings and paintings on stone and recorded some descriptions of consumptives. Their mummified bodies have revealed definite evidence of tuberculosis of bones and joints. In a mummy of the 21st dynasty of Egypt, Pott's disease was established. Tuberculosis as was evident in mummies indicated that, as early 5000 BC, man suffered from it. Babylonian scriptures described tuberculous conditions. Ancient China was not spared from the ravages of tuberculosis. In Chinese literature "Laoping", a disease of the lung that fits in with tuberculosis in its symptoms was mentioned. What is described today as pulmonary tuberculosis was described in India, in 3000 BC. In Rig Veda, which is dated as about 2000 BC, a hymn is consecrated to the cure of "Yakshma" which very much conforms to the present description of consumption or tuberculosis. Susruta described the disease and observed that it was difficult to cure. The fire worshippers of Persia described the disease. In ancient Greece and Macedonia tuberculosis was referred to in some books.

Hippocrates (460-377 BC) also devoted part of his attention to tuberculosis. He opined that attention to the tuberculosis patients was a waste of time and that they were a burden to the state. Half a century later,
Aristotle expressed pity on the unfortunate consumptives. Celsus, in whose lifetime Christ was born and crucified, described early treatment of tuberculosis for which he recommended sea voyages. In the famous library of Leipzig there is a folio which contains information that Jesus suffered from this condition.

Pliny in AD 50 spoke of cold abscess. Aretaeus in Rome was the first to describe haemorrhage from the lungs. Galen (AD 130-200) held that phthisis, as it was called then, was incurable and contagious. He recognized the necessity for rest for the lungs affected by the disease. Vegetious (AD 420) observed that animals were affected by consumption as well as men. The Hebrews prescribed eating of the flesh of tuberculosis animals.

Renaissance and after (1400-1800)

The infectious nature of the disease was described by an Italian Physician Girolamo Fracastoro of Verona (1483-1553). Franciscus Sylvius of Leyden (1614-1672) first employed the term "tubercle" and stated that tubercles were often seen in the lungs of consumptives. Richard Morton (1637-1698) described in his book Phthisiologia, the signs and symptoms of lung tuberculosis after a serious study of the disease for the first time in England. Thomas Willis (1624-1689) separated the tubercles from
ulcers. The great Thomas Sydenham (1624-1689) believed in the virtues of horse riding in the treatment of tuberculosis of the lungs.

Morgagni (1682-1771) dissected bodies of the consumptives and was the first to describe the pathological condition of the lungs. Pierre Desalt (1675-1740) observed that the sputum spread the disease. Benjamin Martin in 1720 foretold the existence of the bacillus one hundred and twenty-two years before its discovery. Gaspard Laurent Bayle (1774-1816) introduced the term tuberculosis for the first time and he traced the relation between pulmonary tuberculosis and tuberculosis of other organs.

William Starke (1741-1771), a physician at St. George Hospital, wrote on pulmonary tuberculosis. In 1761, Leopold Avenbrugger described the art of percussion.

With the dawn of the 19th century new knowledge accumulated. Frenchman Rene Theodore Laennec (1781-1826), who was himself a consumptive, laid the foundations of our knowledge of the aetiology of tuberculosis. In 1819, he invented the stethoscope and described auscultation. From then the fight against tuberculosis is but one step. Laennec and Bayle described tubercles and added to new knowledge. Villemin (1827-1892) demonstrated that tuberculosis was due to a specific agent. Robert Koch
(1843-1910) disclosed to the world on March 2, 1882, his epoch making discovery of the tubercle bacillus. Theobald Smith (1898) isolated the bovine bacillus. Coni (1884) found the chicken tubercle bacillus and the avian bacillus was isolated by Magucci (1890). Rudolf Virchow (1821-1902), the founder of cellular pathology, described the development of caseation in tuberculous tissue and believed that susceptibility to the disease is inherited and not the disease itself. In December 1890, Koch produced tuberculin and described "Koch's phenomena".

Clemens Von Pirquet (1874-1929) described in 1907 his cutaneous reaction and introduced the term allergy to explain the altered reaction. Calmette (1863-1933) and Guerin had been studying the effect of vaccinating animals since 1913. Their Bacille Calmette Guerin (BCG) was described as an attenuated tubercle bacillus after 13 years of subculturing about 230 times. It was only in the year 1924 that BCG vaccination was used in earnest. The Lubeck Disaster (1930) was said to be due to accidental contamination of the vaccine with virulent bacilli. In 1933, Calmette showed that the immunity conferred by BCG lasts for more than five years and that revaccinations are harmless.
In 1913, Hans Much and George Beyeke described the nature of the tubercle bacillus and split up the effects of the various components of the bacilli, i.e. (1) protein, (2) neutral fat, (3) fatty acid lipoid, and (4) toxic factors on tissues.

**Evolution of the modern medical treatment**

William Nisbet (1759-1822) considered alkaline mineral waters, mercury, antimony, cream of tartar and opium useful for the treatment of different symptoms.

Benjamin Rush (1805) in the management of tuberculosis cases divided consumption into three forms, the inflammatory, the hepatic febrile type and the fever with weak pulse type. He recommended Sydenham type of treatment, riding, sailing, running and dancing for chronic cases of tuberculosis.

James Clerk (1788-1870) laid great stress on climate though he was pessimistic about the curability of the disease. The intensity of therapy varied widely from bleeding, purging, blistering and induced emesis to palliative measures with some recognition of the value of outdoor life.

**The sanatorium movement**

It was George Bodington (1799-1882) of England who published a monograph in 1840, suggesting construction of hospitals in the country for consumptives. This was looked upon as the beginning of the sanatorium movement. Herman
Brehmer (1826-1899) in Germany submitted his thesis in 1853 on the laws concerning the beginning and progress of tuberculosis of the lung. He advocated rest treatment for tuberculosis and, as for exercise, his recommendation was moderation. In 1859, he set up his sanatorium in Goerbersdorf in the mountains of Silesia and prescribed his patients balanced diet, fresh air, baths and regulated exercise. This movement spread through his students and other physicians.

In U.S.A., Trudeau (1848-1915) broke down with tuberculosis. His improvement in the mountain resort encouraged the treatment of the tuberculous patients in sanatoria located in hill stations. Thus, the Adironback Cottage Sanatorium started as the "Little Red" Cottage Trudeau, New York, in 1884, which proved to be the model for the other sanatoria in U.S.

Chemotherapy in tuberculosis

Cod Liver Oil had been used in 1822 by Schenk for scrofula and for tuberculosis by Bennet in 1841.

As far back as the time of Parecelsus, gold was given for consumptives. Koch in 1890 found gold cyanide 1/2,000,000 lethal to tubercle bacillus in vitro and of no value in vivo. Mollgaard (1924) reintroduced gold in
the form of a thiosulphate as Sanocrysin. In the begin-
ing it was considered chemotherapeutic, then catalytic
and lastly as an activator of the immunological processes
through the reticuloendothelial system. Other forms of
gold i.e. Solgenal and Myocrisin were introduced with no
better results.

Copper salts were used in 1894 in France. Van Linden
and others used copper for the treatment of Lupus in 1912.

In 1927, Perey Moxcy introduced an antimony prepara-
tion to be used through the intramuscular route.

In the modern period, the search for chemotherapeutic
drugs was activated by Domagk’s introduction of Prontosil.
Promin, Promizole, Sulphetron, etc. have been found useful
against the tubercle bacillus. All these were found to be
extremely toxic and given up.

A new era of chemotherapy dawned in 1944 when Schatz
Bugie and Waksman published their paper on streptomycin
and its action on Mycobacterium tubercle bacillus and
related organisms. Feldman and Hinshaw tested its action
on animals and man, as they tried promizole before. By
1947, the drug was available in quantity and came into
general use by 1949.
Since the introduction of streptomycin, several synthetic antimicrobials and antibiotics have been discovered from time to time. They are, among others, para-amino salicylic acid by Lehmann (1946), isonicotinic acid hydrazide by Grunberg et al. (1951), pyrazinamide by McCune (1955), ethionamide, cycloserine, kanamycin, viomycin, capreomycin, ethambutol and rifampicin.

Lessons of history tell us that the need of the hour is the effective treatment of cases with chemotherapy by combination of drugs for sufficiently long time, through periodic laboratory tests for sensitivity of drugs to the tubercle bacillus, least drug resistance become a great social danger. One remarkable effect of chemotherapy is the protection afforded to surgical procedures like lung resection. Therapy has marched a long way from the days of empirical drug giving to specific chemotherapy and surgical excision. The use of rifampicin in combination with isoniazid etc., as a primary drug has revolutionized the therapy of tuberculosis and shortened the duration of treatment.

A history of tuberculosis control in India

The first open-air sanatorium for the treatment and isolation of tuberculous patients was founded in 1906 in Tilaunia near Ajmer by a Christian Mission. It was
intended mainly for girls from schools and orphanages connected with the missions in North India. An institution in Almora in the Himalayas for tuberculous women was also started by Christian Missions in 1908 and about the same time a small sanatorium for women and girls at Pendra Road in the then Central Provinces was also opened. In the Union Mission Tuberculosis Sanatorium, Madanpalle, which was started in 1908, pioneer work was done by Dr. Louisa Hart, a missionary working in Madanpalle town. The Union Mission Tuberculosis Sanatorium was shifted in 1915 to Arogyavaram near Madanpalle with Dr. C. Frimodt-Moller as its Medical Superintendent.

A number of private societies were started simultaneously. The first sanatorium outside Mission auspices was opened in 1909 at Dharampore in Simla Hills due to the benefactions of some Bombay philanthropists, under the management of consumptives Homes Society of Bombay.

The first sanatorium under government supervision called the King Edward Sanatorium, was opened at Bhowali in 1912 with funds collected in that Province in memory of King Edward VII. There have been also individual efforts by private individuals. Dr. R. B. Billimoria opened a Sanatorium at Poona in 1912 which two years later moved to its present site in Panchgani, Maharashtra State.
Other sanatoria, opened at the same time, were Turner Sanatorium in Bombay and the Deolali Sanatorium.

History and development of International cooperation in the conquest of tuberculosis

With the birth of WHO in 1948, a global approach to tackle the problem of tuberculosis was made. BCG vaccination was given top priority and was taken up in collaboration with UNICEF and International Tuberculosis Campaign (ITC).

Before the Second World War, tuberculosis was considered a problem for each nation's health authorities and there was comparatively little work for International organizations. The League Nations had collected for statistics on morbidity and mortality. The International Union Against Tuberculosis is perhaps the most important organization which organizes International Conferences where delegates from different countries exchange opinions. Towards the end of the Second World War, the United Nations Relief and Rehabilitation Administration (UNRRA) sent doctors and nurses to help in organizing tuberculosis control in countries like Greece and China.

During the second 10 years of the WHO (1958-1967) the technical and operational knowledge acquired in the first 10 years permitted the planning of comprehensive
National Tuberculosis Programmes particularly in developing countries where expenditure on tuberculosis needs to be adjusted to the very limited resources. The epidemiological pattern of tuberculosis in the developing countries emerged from a series of WHO assisted surveys in more than 20 countries in the Asia and Africa. It was found that age/sex distribution of the disease was very similar in both developed and developing countries and the difference between the urban and rural distribution was smaller than had been thought. WHO assisted in evolving new therapeutic regimens and establishing the efficacy and feasibility of out-patient and domiciliary treatment of tuberculosis.

The WHO is functioning as a coordinator of a global tuberculosis programme by assisting National Tuberculosis Programmes, giving technical information, in development of standards, with regard to nomenclature and classification, products, methods and research. The International voluntary organizations and bilateral agencies have been functioning with the general framework of broad objectives of the programmes.

In the words of Dr. Candau, the Director-General of WHO (1969), "we must work for the world-wide cooperation of all Nations in our endeavours - for a full and not merely a partial participation in our common efforts-for
therein lies the way to universal peace, prosperity and health."

The 9th WHO Expert Committee on Tuberculosis observed -

"The Eighth WHO Expert Committee report had offered for the first time the concept of comprehensive tuberculosis control programme on a country-wide scale. Consequently in many countries, tuberculosis from being a clinical speciality has become a widely applied community health activity. The committee recommended that the National Tuberculosis Programme should be permanently adapted to the demands of the population and integrated in the community health structure. The components of the programme are case-finding and treatment and BCG vaccination. The importance of evaluation, research and the role of voluntary organizations as a complement and support to the Government activities were stressed."

Tuberculosis in a tropical environment

Tuberculosis in many tropical countries differs from that generally seen in western countries in the severity of the infections, which often present at a much more advanced stage. The large population of bacilli result in haematological spread to a wider spectrum of organs causing for example, meningitis, osteitis or lymphadenitis.
Extensive tissue damage is more common during both the infective and healing phases. Children under 5 years are more frequently involved. Malnourished children seem to have a deficient cellular immune system which makes them susceptible to infectious diseases, particularly tuberculosis. Factors such as malnutrition, isolation and bed rest are no longer thought to be significant in determining the outcome of treatment (Ramakrishnan et al., 1966) in comparison with the strength and duration of the chemotherapy regimens and the regularly with which these are taken (Fox, 1977). Considerations which apply in the choice of a regimen for pulmonary disease in a tropical country are illustrated by the following three examples.

1. Rifampicin and isoniazid daily for 9 months with an initial supplement of streptomycin is a powerful bactericidal regimen, highly effective but expensive and hence beyond the financial resources of many of the poorer tropical countries.

2. Streptomycin, isoniazid and para-aminosalicylic acid (PAS) for at least 12 to 18 months is also effective but the longer duration of therapy (as it is a weaker combination) and the unpleasant side effects of PAS reduce patient compliance. Moreover, in many developing countries, the daily administration of streptomycin almost invariably
requires hospital admission for the duration of treatment.

3. Thiacetazone and isoniazid eliminate the bacilli from the sputum after 12 months treatment in over 80% of cases. The addition of streptomycin during the first 2 months improves the rate of sputum conversion to 90%. Relapse, however, is frequent. Thiacetazone is a weak, bacteriostatic drug whose main action is to prevent isoniazid resistance. The combination is, however, very cheap, compact and stable in tropical conditions and on these grounds is widely used; but in some population (e.g. Filipioos), it is very poorly tolerated and is unacceptable for use.

Treatment of pulmonary tuberculosis (in Baroda district)

It is known that implementation of National Tuberculosis Control Programme has not made the desired impact on the problem of tuberculosis in the last two decades of its implementation. The main reasons for poor impact are poor case detection and poorer case holding. 70-75% population is rural. The problem of tuberculosis is more or less the same in urban and in rural population; hence 70-75% of tuberculosis patients are in rural areas. Of these only 50% go to the nearest medical relief centres, viz. P.H.C., P.H.U., H.U., Government dispensaries, Panchayat dispensaries and private practitioners both
allopathic and indigenous. Data of various centres show that contribution of peripheral health institutions under general health services, in case detection varies from 8% to 28% (only 20% P.H.I.'s contribution is more than 20%).

According to experience at Padra, rifampicin can be handled safely by Para-medical workers under field conditions. Administration of rifampicin may be left to the patients as TZN and INH are left to self-administration. The rhythm of self-administration may be verified at each monthly collection of drugs. Rifampicin is not likely to be more misused than streptomycin, as supply in number and the cost remains the same.

It is very strongly recommended that rifampicin should be introduced to strengthen the national regimen without increasing the cost and any further delay. Patel A.G. (1982) is of the opinion that under the present circumstances streptomycin should be gradually and completely replaced by rifampicin.

Statement below shows the cure rate, adverse reaction and efficacy under programme condition -
### Regimens used in National Tuberculosis Control Programme

<table>
<thead>
<tr>
<th>Treatment regimen</th>
<th>Cure rate % (trial)</th>
<th>Adverse reaction</th>
<th>Overall efficacy under programme condition</th>
<th>Cost in Rs.</th>
<th>Rural/urban</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>65%</td>
<td>10%</td>
<td>50%</td>
<td>70/-</td>
<td>Rural and urban</td>
</tr>
<tr>
<td>R2</td>
<td>80%</td>
<td>7%</td>
<td>60%</td>
<td>250/-</td>
<td>Urban</td>
</tr>
<tr>
<td>R3</td>
<td>70%</td>
<td>10%</td>
<td>50%</td>
<td>400/-</td>
<td>Rural and urban</td>
</tr>
<tr>
<td>R4</td>
<td>60%</td>
<td>1%</td>
<td>40%</td>
<td>40/-</td>
<td>Rural</td>
</tr>
<tr>
<td>R5</td>
<td>80%</td>
<td>12%</td>
<td>60%</td>
<td>210/-</td>
<td>Urban</td>
</tr>
</tbody>
</table>

**Not advisable**

| R5                | 80%                 | 12%              | 60%                                       | 210/-      | Urban     |

Treatment of tuberculosis is long drawn out and irregularity in drug intake is often encountered. About 30% of patients do not come for second collection of drugs and 50% do not come to collect drugs by the end of 3 months. On an average about 10% of patients complete treatment and...
the performance of reputed institutions do not go beyond 30%. Poor compliance of patients is the major cause of failure to make any impact on the tuberculosis problem in this country.

Shortening the duration of treatment, therefore, with the use of a potent drug like rifampicin will not only improve patient's compliance but will also reduce the number of chronic patients. Rifampicin forms a most powerful combination with isoniazid. Its bactericidal and sterilizing properties are unique (Fox, 1979). It is active on intracellular and extracellular organisms at all pH levels, is freely distributed in body fluids and starts acting within two hours of administration. Its MIC value is 0.2 ug/100 ml. While the highest blood level varies from 10-20 ug/100 ml (Grosset, 1978). The drug can be used intermittently thrice, twice or once a week with other antitubercular drugs resulting in high acceptability and low relapse rates (Verbist, 1972; Eule, 1973; Fox and Mitchison, 1975; Kreis et al., 1977; Alouch, 1978; Anastasatu, 1979; Tripathy, 1979; Krishnaswamy, 1982).

Rifampicin when given daily is very expensive and beyond the reach of developing countries. Successful efforts have been made to reduce the cost of rifampicin treatment by giving intermittently. It has been found to
be more effective in guinea pigs when given at longer intervals (Mitchison and Dickinson, 1978; Hong Kong Chest Services/British Medical Research Council, 1979).

**Microbiological basis for short-course therapy with rifampicin**

Much more knowledge has since been acquired about the way in which rifampicin distinguishes itself from all other commonly employed antituberculous agents. From the investigations of Grosset (1978) and co-workers at the Pasteur Institute and the experimental *in vivo* and *in vitro* work of Mitchison and Dickinson (1978) as well as the results of several large-scale clinical studies, it now seems reasonable to assume that the mycobacterial population in human tuberculosis - as in the case of murine tuberculosis in the mouse - is made up of four subpopulations which are distinguished chiefly by their different speeds of growth (Mitchison, 1979). The main group - estimated at $10^7$ to $10^9$ organisms - consists of the continuously and relatively rapidly growing population A, which finds favourable growth conditions in the liquified content of cavities with a good oxygen supply and at a neutral pH (Grosset, 1980). This group is destroyed by the bactericidal action of isoniazid, rifampicin and streptomycin. At the other end of the growth scale is group D.
with its dormant organisms, which are probably not susceptible to any chemotherapeutic agent (Grosset, 1978; Mitchison, 1979). Groups B and C comprise organisms which are located either in the acid intracellular environment of macrophages (group B) or under low oxygen pressure in solid caseous masses (group C) and which, owing to the unfavourable growth conditions, multiply only slowly or during short intermittent periods. It is against these comparatively sparse but, in the event of their persistence, dangerous groups (which can cause relapses if and when the body's resistance becomes lowered) that the "sterilizing" action of rifampicin and pyrazinamide is directed.

In contrast to rifampicin, pyrazinamide is able to act only on organisms in an acid milieu (group B); the lower the pH (5.6 or less), and hence the lower the bacterial growth rate, the more effective pyrazinamide proves.

On the other hand, rifampicin is the only antituberculous agent capable of destroying organisms in group C (Fox, 1981). This subpopulation is for the most part dormant, and therefore remains insusceptible even to bactericidal agents; occasionally, however, it becomes metabolically active, even though perhaps for no more than a few hours. Such brief proliferation phases are sufficient
to enable rifampicin — though not isoniazid — to destroy the organisms (Fox, 1981), which already begin to die off after only 15-20 minutes' exposure to rifampicin (Mitchison, 1979). The sterilizing action of rifampicin is thus attributable to the extremely rapid onset of its bactericidal effect, which can also be impressively demonstrated in vitro.

In addition, rifampicin also exerts a bactericidal effect on intracellular tubercle bacilli (group B), its ability to penetrate phagocytes probably being due to its excellent liposolubility (Mandell and Vest, 1972; Lobo and Mandell, 1973; Mandell, 1973; Ezer and Soothill, 1974; Samson and Lapointe, 1977; Soldberg and Hellum, 1978). In a study carried out with alveolar macrophages, the concentration of rifampicin built up in these macrophages during the two-hour incubation period was twice as high as in the extracellular space surrounding them, whereas the intracellular concentration of isoniazid was equivalent only to 50-80% of the extracellular concentration (Johanson et al., 1980). Similar findings have been reported by Harf et al. (1982). Since the bacilli appearing later in solid caseous material are recruited from the intracellular population, destruction of these intracellular organisms by pyrazinamide, rifampicin and isoniazid may be expected to speed up sterilization and also to reduce the relapse
rate after the completion of chemotherapy (Grosset, 1980) - as has in fact been confirmed in numerous therapeutic studies.

In the light of knowledge acquired to date it can thus be concluded that both isoniazid and rifampicin exert a strong bactericidal action against continuously and relatively rapidly multiplying tubercle bacilli (Mitchison, 1979; Fox, 1981); both drugs are also clearly effective against the slowly multiplying bacilli within macrophages, although they do not measure up to pyrazinamide in this respect; only rifampicin, however, is capable also of destroying those organisms which multiply slowly or intermittently in caseous masses (Grosset, 1980), and for this reason its "sterilizing" action surpasses that of pyrazinamide, both in scope and in importance.

These facts provide the theoretical foundation for short-course chemotherapy (Fox, 1978; 1981). For this form of therapy, the most effective basic combination - which, depending on the therapeutic objectives envisaged, may be supplemented by further drugs - is isoniazid plus rifampicin with the initial addition of pyrazinamide.
Microbiological basis for intermittent therapy with rifampicin

That rifampicin fulfils one important prerequisite for intermittent therapy has been confirmed by the following experimental finding (Mitchison and Dickinson, 1971; Dickinson et al., 1972; Dickinson, 1974).

When a culture of M. tuberculosis is exposed to an unvarying concentration of rifampicin on several consecutive days for a limited time at 24-hour intervals, the drug's bactericidal effect decreases at the second and at each subsequent exposure in comparison with the first exposure. If, on the other hand, the interval between two exposures is prolonged to such an extent that the organisms begin to show renewed signs of growth - i.e., at the end of the "lag period" - then each subsequent exposure produces a bactericidal effect equivalent to that of the first exposure. The lag period, or duration of growth inhibition after the end of the exposure (when the drug is then washed out), is relatively short for rifampicin, and it increases only slightly when the exposure time is lengthened several fold.

The bactericidal action of rifampicin sets in immediately after the start of exposure, but does not increase in direct proportion to the exposure time and
concentration. In this *in vitro* model, therefore, rifampicin acts in a fundamentally different way from isoniazid, whose efficacy during intermittent exposure depends on the length of the lag period (which in turn, depends very much on the duration of exposure and on the concentration) and whose bactericidal action is slow to take effect. From this it can be concluded that each single dose of rifampicin, administered every two to three days in an intermittent regimen, exerts a full-blown bactericidal action, even though the dose may be equal to or only slightly above that administered daily; in the case of isoniazid, on the other hand, it seems rational to aim at maintaining relatively high serum concentrations for as long as possible.

In keeping with these *in vitro* observations, *in vivo* studies on mice and guinea pigs at the Pasteur Institute have also shown that the combination of rifampicin plus isoniazid is superior to other forms of two-fold combination when administered intermittently (Grumbach et al., 1969; Le Lirzin, 1973).

**Undernutrition and drugs pharmacokinetics**

One of the important environmental factors which can alter drug metabolism and kinetics is the nutritional status of an individual. Drugs, which are neither curative nor preventive measures for the problem of malnutrition, are
nevertheless being widely used in all developing countries for treating infections and illnesses which co-exist with malnutrition. In addition, the chemical environment of man is ever changing and today there is a chemical overloading of nature. Drugs and chemicals are termed as "xenobiotics" and are handled in a similar way in the human body. The pharmacological/toxicological activities of these xenobiotics are to a great extent therefore determined by the capacity of the individual to metabolize them. Modern trend in drug therapy is to try to individualize therapy to obtain maximum therapeutic response with minimal side effects. Clinicians are interested in titrating the dose of the drug to the desired effect. There is a keen awareness at present about the safety of drugs and chemicals not only among scientists but also among the general population.

Malnutrition is a complex problem with its multiple inter-relationship with a host of other environmental factors. There is now increasing awareness of the interplay between nutrition and drug metabolism. However, most of the studies in this area are still confined to experimental situations and there are very limited data on human populations.

The therapeutic response of an individual to a drug is influenced by a variety of factors which include the rate and extent of absorption, utilization, elimination and
tissue response. In addition, a number of internal and external factors, inherited and acquired differences in metabolic conversions modify the response.

One of the important environmental influences which may alter drug distribution and disposition is nutritional status. Malnourished subjects in developing countries are often treated with a variety of drugs. While there is some information regarding the role of drugs in modifying nutritional status, there are little data on how the nutritional status of an individual modifies drug kinetics which in turn has several therapeutic implications. However, there are reports which indicate that the liver microsomal enzymes concerned in detoxification process of drugs are considerably affected by various nutritional constraints.

Pathophysiological changes in undernutrition altering pharmacokinetic parameters

Drug metabolism may be defined as the biological fate of either a drug or a xenobiotic in terms of absorption, distribution, biotransformation and excretion. The therapeutic response of an individual to a drug cannot always be predicted with accuracy. A number of pharmacokinetic parameters differ significantly and are subject to variation due to internal and external variables. Deficiencies of several nutrients such as proteins, calories, vitamins and
minerals, result in a number of pathophysiological changes which may have a direct bearing on drugs and their kinetics.

The changes which are likely to lead to pharmacokinetic alterations are the following: Changes in gastrointestinal tract, alterations in body composition and body fluids, reduction in plasma and tissue proteins, hepatic and renal involvement, changes in the hormonal profile and metabolic alterations. These changes have wide repercussions on a number of other parameters and may affect many processes in drug metabolism.

Studies on chloramphenicol (Mehta et al., 1975)

Chloramphenicol is metabolized by the microsomal enzyme system. A recent report indicates that chloramphenicol is not metabolized in malnourished children to the same extent as in normal children. Data in undernourished subjects indicated that the microsomal enzymes are in an induced state. Hence a study was undertaken to estimate the $C_{min}$ at steady state in undernourished subjects. There were no significant differences between normal and undernourished subjects even in the $C_{min}$ concentrations. This apparently means that the pharmacokinetic parameters may not be different in undernourished population, even though chloramphenicol is metabolized by microsomal enzymes. These results are applicable to undernourished adult subjects. However,
the involvement of liver in malnutrition is more often severe in children than in adults. Therefore, the age of the patient may play a significant role in determining the various pathophysiological changes and consequent changes in other parameters.

Studies on tetracyclines (Shastri, R.A. and Krishnaswamy, K. 1976)

The tetracycline group of antibiotics is commonly used in treating a wide variety of infections in our country. Tetracycline does not undergo any biotransformation and is excreted unchanged in the urine. However, it is bound to plasma proteins and only the free fraction is eliminated in the urine. Therefore, the kinetics of tetracycline was studied in a group of undernourished subjects in whom the plasma albumin concentrations were significantly low. These subjects had also weight deficit as judged by the weight/height index. Tetracycline in doses of 10 mg/kg body weight was administered both by oral and intravenous routes and pharmacokinetic parameters like elimination rate constant, plasma half-life, volume of distribution and total body clearance rate were calculated. Urinary excretion within 24 hours was determined and plasma protein binding was estimated.
The plasma half-life of tetracycline hydrochloride was determined in groups of well nourished and undernourished subjects employing two dosage schedules and two routes of administration, namely oral and parenteral. Protein binding of the drug was estimated and relative volume distribution was calculated. The results indicated that in undernourished subjects, the half-life of tetracycline hydrochloride, protein binding and relative volume of distribution were all significantly low as compared to well nourished subjects. The elimination rate was considerably higher in the undernourished groups, which accounted for the shorter half-life. These data suggest that to maintain adequate serum concentrations of the drug in undernourished subjects, tetracycline needs to be given at more frequent intervals.

Studies on antipyrine (Krishnaswamy and Naidu, 1977)

Drugs and chemicals are detoxified by microsomal enzymes and the more polar and water soluble derivatives so formed are excreted from the body. Individuals differ in their genetic potential to handle drugs and several environmental factors are known to modify the microsomal enzyme system. In animals, it has been found that a number of macro and micro nutrient deficiencies result in an alteration of drug metabolism. Similar studies in man are scanty.
The microsomal enzyme system in man was therefore investigated using antipyrine half-life and clearance rate as indices of drug metabolism. In addition to undernourished subject, adult patients with severe protein-calorie malnutrition (nutritional oedema) were also included.

The results of this study are given. The rate of decline of antipyrine concentrations in plasma was significantly different between groups. The half-life of antipyrine in normal smoking population was significantly shorter with a higher metabolic clearance rate as compared to non-smokers. In the group of undernourished subjects both the plasma half-life and metabolic clearance were significantly different from values seen in non-smoking normal adults. The clearance rate was significantly higher but was similar to that of smokers. Significant differences in the clearance were observed when it was adjusted for body weights. These observations indicate that both in normal smokers and in undernourished subject, microsomal enzymes are in an induced state. On the other hand, in severe nutritional oedema subjects, the plasma half-life was significantly prolonged as compared to undernourished subjects and the metabolic clearance rate was significantly decreased. However, the values obtained were within the normal range. In these subjects there are two opposing factors. Although the mean values obtained appeared to be
similar, more than half the number of subjects studied i.e. (8 to 15) had values below the mean value of normals and half of them had values which were below the lowest obtained in the normal group. These changes indicate that severely malnourished individuals may be at a greater risk of accumulating potent/toxic drugs which are metabolized by MFO. That there is liver damage in nutritional oedema is supported by the observation that many subjects had elevated y-glutamyl transpeptidase activity, which is now considered to be a valuable indicator of hepatic damage. However, the undernourished subjects appear to have an intact microsomal enzyme system, which can be stimulated by environmental factors such as smoking, alcohol and probably ingestion of pesticides.

Studies on streptomycin (Prasad and Krishnaswamy, 1977)

Since tuberculosis is widely prevalent in our country and streptomycin is the most commonly used aminoglycoside, investigations were conducted to study the pharmacokinetics, when streptomycin was given by the intramuscular route. No differences were observed in any of the parameters, between well nourished and undernourished subjects.

Studies on sulfadiazine (Shastri and Krishnaswamy, 1978)

Sulfa compounds are still widely used in our country for a variety of infections. Sulfadiazine is also bound to
the extent of 50-55% and is mainly acetylated in the liver by a cytosol enzyme. The acetylated product is usually associated with side effects. Sulfadiazine is administered every 8 hours, to maintain the blood concentrations above 10 mg% - the effective therapeutic level. Kinetic studies were therefore undertaken to determine the various parameters and steady state levels in undernourished subjects. The elimination rate constant was found to be significantly higher in undernourished subjects and consequently plasma half-life shorter, both on oral and intravenous administration of sulfadiazine. The volume of distribution of the drug was essentially similar with a significant increase in metabolic clearance in undernourished subjects. Though the urinary excretion of the total drug was similar, the acetylated fraction was significantly higher in the undernourished. This was due to the fact that plasma protein binding of the drug was significantly lower (40.6%) as compared to that in well nourished subjects (54%), there being more free drug for acetylation. When sulfadiazine was administered at a dose of 80 mg/kg weight at 8 hourly intervals, the blood concentrations at 48 and 72 hours tended to be lower in undernourished subjects due to faster metabolic clearance. However, even these concentrations were in the therapeutic range and hence despite significant differences in plasma protein binding and metabolic clearance, there appears to be no need to alter the dosage schedules of sulfadiazine in the undernourished subjects.
Protein calorie malnutrition and animal models

In the past, many investigators accepted the need for animal models of protein calorie malnutrition (PCM). There has, however, been doubt concerning the resemblance of these models to human syndromes and the validity of applying results obtained in animals. Recent models of PCM closely resemble human syndromes such as kwashiorkor and marasmus, and in many cases the results obtained have been found to apply in man. It would thus seem reasonable to use suitable animal models of PCM to clarify further the pathophysiology of human PCM, especially where human studies would be hazardous or impossible.

1. It has been shown in rats that certain proteins have poor biological values - these poor values also apply in man (Brock, 1961; Njaa, 1965).

2. The existence of a direct relationship between the net protein utilization of various diets and the calorie levels of the diet fed suggested by animal work and has been confirmed in man (Miller and Payne, 1961).

3. Changes in body water values, which have been shown to occur in human and experimental kwashiorkor, are essentially similar while the "reversion" to an earlier stage of body composition found in animals (Platt, 1964) is also found in man (Brock and Hansen, 1965).
4. In the field of haematology, animal models of PCM have been found to have an anaemia (with normal serum iron levels) that does not respond to iron but responds to protein. Similar changes occur in human disease (Brock, 1961).

5. Changes in the serum proteins, especially in the serum albumin concentrations, have long been regarded as the most convenient index of man's protein nutritional status and a definite correlation has been found between the severity of the disease in man and the serum albumin level (Schendel, 1962). This correlation has been confirmed in animals fed diets containing various levels of protein. Much work has been done on the kinetics of albumin metabolism in children with kwashiorkor and in adult volunteers put on low protein diets. This work has led various authors to postulate that reduction of the dietary protein level leads to reduced synthesis of albumin. This drop in albumin synthesis results in a fall in the plasma albumin concentration. The diminution in albumin pool size, it was postulated, led to a lowering of the catabolic rate in an attempt to conserve albumin. This could only be tested in animals and has indeed been substantiated in rats fed a protein-free diet (Kirsch, 1968).
6. The gastrointestinal tract has for a long time been the subject of considerable attention in PCM and similar changes in intestinal morphology have been found in both animals and man (Platt, 1964). However, very little disturbance of absorption of protein has been found in nitrogen balance studies in animals or in man (Hansen, 1956). Amino acid transport experiments have thus been carried out in loops of gut from rats with PCM and these have given normal figures thus supporting the findings of nitrogen balance studies in children (Kirsch, 1968).

7. Rats fed a poor quality protein diet showed a reduction in pancreatic enzymes. This deficiency of pancreatic enzymes has been also demonstrated in children suffering from PCM (Barbazat, 1967).

8. Less encouraging results have been obtained from animal models in the study of endocrine disorders where workers have found changes suggestive of hyposecretion, inter alia, of growth hormone (Strebnick and Nelson, 1962). These conclusions were based on histological changes and on measurement of the amount of hormone present in the gland. High levels of growth hormone are now known to be present in the blood of some children suffering from PCM (Pimstone et al., 1966).
Although animal models have been extensively used in the study of protein-calorie malnutrition there is controversy regarding the validity of extrapolating results from animals to man. There is a need for experimental models of PCM.

PCM in man is invariably complicated by deficiencies of vitamins, minerals, and perhaps trace elements as well as intercurrent infections and infestations. These alter, in endlessly different patterns, the basic clinical and metabolic pictures arising from different pattern of community diets in various parts of the world. The common factor in all these syndromes is inadequacy of dietary protein so that the use of an experimental animal fed a diet deficient solely in protein has obvious advantages. All experimental studies on the human syndromes are influenced by the urgent need for energetic therapy and this is not the case in the experimental model. In addition, animal models may be produced in large numbers and individual variation can be kept to a minimum. For these reasons animal models of PCM are highly desirable.

Rifampicin is the latest but welcome addition to the plethora of drugs used in the treatment of tuberculosis. Rifampicin is active against most of the gram positive as well as gram negative organisms, but with the emergence of resistant bacteria and availability of other potent
antibiotics, the drug is mainly held in reserve for the treatment of tuberculosis. The antibiotic is also reported to be useful in leprosy and may inhibit growth of certain types of viruses.

The place of rifampicin in the spectrum of antituberculous drugs now seems to be established. The antibiotic has been recommended with other antituberculous drugs in the standard and intermittent chemotherapeutic regimens for the initial or retreatment of tuberculosis as shown in following tables -

**Standard daily regimen (2 years duration)**

<table>
<thead>
<tr>
<th>Initial phase (3 months)</th>
<th>Continuation (18 to 24 months)</th>
<th>Dosage drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPI</td>
<td>PI</td>
<td>S - 0.75 - 1 gm</td>
</tr>
<tr>
<td>SRI</td>
<td>RI</td>
<td>P - 10-12 gm</td>
</tr>
<tr>
<td>SEI</td>
<td>EI</td>
<td>R - 450 or 600 mg</td>
</tr>
<tr>
<td>STI</td>
<td>TI</td>
<td>E - 15 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T - 150 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I - 200-300 mg</td>
</tr>
</tbody>
</table>

*S* - Streptomycin  
*P* - PAS  
*B* - Ethambutol  
*I* - Isoniazid  
*R* - Rifampicin  
*T* - Thiacetazone
Intermittent regimen

<table>
<thead>
<tr>
<th>Daily phase (3 months)</th>
<th>Phase (18-24 months) with twice weekly</th>
<th>Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPI</td>
<td>S2I2</td>
<td>S - 0.75-1 gm</td>
</tr>
<tr>
<td>SRI</td>
<td>S2I2</td>
<td>I - 15 mg/kg</td>
</tr>
<tr>
<td>SEI</td>
<td>E2I2</td>
<td>E - 50 mg/kg</td>
</tr>
<tr>
<td>STI</td>
<td>S2I2</td>
<td></td>
</tr>
</tbody>
</table>

Chemistry of rifampicin

Rifampicin is a semisynthetic derivative of a macrocyclic antibiotic rifampicin-B. There are three main steps leading to the formation of rifampin, a semisynthetic antibiotic exhibiting greater oral absorption and slower biliary elimination than its parent molecule rifamycin B (Maggi et al., 1966). Rifamycin B is preferentially produced in N. mediterranea fermentation cultures supplemented with sodium diethylbarbiturate (Margalith and Pagani, 1961). In oxygenated aqueous solutions of purified rifamycin B, pH 3-4, two rifamycins with comparatively high antibacterial activity are formed, namely rifamycin O and rifamycin S (Oppblzer and Prelog, 1973). Rifamycin SV is derived from rifamycin S by mild reduction with ascorbic acid. Finally, rifampin is obtained by reacting 3-formylrifamycin SV with 1-amino-4-methyl-piperazine in tetrahydrofuran (Maggi et al., 1966; Gadat et al., 1975).
In solution, rifampin behaves as a zwitterion. The acidic function ($pK_a = 1.7$) is associated mainly to its $C_1$ and $C_8$ hydroxyl groups, while the basic function ($pK_a = 7.9$) is related to the piperazine-nitrogen group (Assandri et al., 1977).

Chemical structure of rifampin, the full chemical name is: 3- (4-methyl-1-piperazinyl)imino methyl -5,6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8- N-(4-methyl-1-piperazinyl)formimidoyl-2,7 (epoxypentadeca 1,11,13 trienimino)-naphtho 2,1,-b furan-1, 11-(2H)-dione-21-acetate. The abbreviated chemical name is: 3-(4-methyl-1-piperazinyl-imino-methyl)-rifamycin SV.

Solubility of rifampin in various organic solvents*

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl alcohol</td>
<td>39.0</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>5.0</td>
</tr>
<tr>
<td>Acetone</td>
<td>14.0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>399.0</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>216.0</td>
</tr>
<tr>
<td>N-hexane</td>
<td>0.43</td>
</tr>
</tbody>
</table>

* Data provided by Dow-Lepetit, Milan, Italy  
(Kenny and Strates, 1981).
Rifampin is a slightly soluble in water and its solubility is temperature- and pH-dependent. Solubility at 25°C is approximately 10 g/100 mL at pH 2.0, 0.4 g/100 mL at pH 5.3 and about 0.28 g/100 mL at pH 7.5 (Puresz et al., 1970; Boman et al., 1975). The solubility of rifampin in various organic solvents is given in the table above.

Aqueous solutions of rifampin are relatively unstable. At acid pH (......2.3) part of the rifampin in solution is hydrolyzed to 3-formyl-rifamycin SV and 1-amino-4-methyl-piperazine. At alkaline pH and in the presence of atmospheric oxygen rifampin is slowly oxidized to a rifampin-quinone (Maggi et al., 1966). Oxidation is inhibited in the presence of reducing agents. More specifically, the stability of aqueous solutions of rifampin at alkaline pH is increased by the addition of sodium ascorbate (......200 ug/ml) (Maggi et al., 1966).

Solutions in dimethylsulfoxide (10 mg rifampin/ml DMSO) at 15°C have been reported to remain stable for at least 3 months (Karlson and Ulrich, 1969). Lyophilized preparations of rifampin for intravenous administration are stable for a period of at least 2 years before reconstitution. Suspensions of 1% rifampin powder in simple syrup (sucrose 85% w/v) stored at 2-8°C were found stable by microbiological and chemical assays for at least 6 and 7
weeks, respectively. Degradation products include 3-formyl-rifamycin, 25-O-desacetyl rifampicin and rifampin-quinone.

**Fundamental pharmacokinetic properties of rifampicin**

**Absorption**

Gastric pH is of importance in the process of absorption of rifampicin in man. Acidification of gastric juice with histamine results in serum concentrations twice as high as those observed after alkalisation with sodium bicarbonate (Velo and Vettori, 1968). These findings are consistent with those of Riess (1968) who found that marked variations in serum rifampicin levels occurred if the drug was given on an empty or full stomach. As a result of these studies and of those of Hussels (1969) and others, it is recommended that rifampicin be administered on an empty stomach. Under these conditions, the absorption of rifampicin is rapid and practically complete. In single subject studies, an oral dose of 300 mg $^{14}$C-rifampicin administered preprandially together with a bolus intravenous dose of 300 mg $^{3}$H-rifampicin gave nearly identical serum concentration curves and similar AUC$^{0-24}$ hour values. The so-called 'first pass' hepatic effect for the antibiotic during its initial passage through the hepatoporal system and transfer to bile can probably be regarded
as the most important single factor affecting the time
course of rifampicin concentrations in the blood of the
systemic circulation.

**Blood concentrations**

**Single dose administration**

Peak concentrations of the order of 10 ug/ml can be
expected to occur 24 hours after the administration of a
standard dose of 600 mg and, as can be seen from the data
reported by Riess, 1968; increasing the dose of rifampicin
does not result in a proportional increase of the peak
concentrations. Deviation from linearity has also been
observed for the area under the serum concentration-time
curve from 0 to 12 hours. This phenomenon can probably be
related to the marked 'first pass' hepatic extraction of
rifampicin and achievement of a transport maximum (Tm) in
bile at doses lower than 600 mg.

The biological half-life of the antibiotic has also
been found to increase significantly with the administered
dose. The mean half-life of elimination increases from
2.6 hours for a 300 mg dose to 3.3 hours for a 600 mg dose
to 5.1 hours for a 900 mg dose (Acocella et al., 1971).
A significant increase of the half-life with the dose,
expressed in terms of mg/kg body weight, was also found by
Curci et al. (1970). An intravenous preparation has been
licensed and is being investigated in United States.
In dogs doses as low as 0.625 mg/kg give serum levels which, although not excessively high, nevertheless last 36-40 hours. At the dose of 2.5 mg/kg, the serum concentrations are much higher and concentrations around 1 ug/ml are still present after 32 hours. Detectable blood levels are found even after 56 hours, demonstrating a very slow elimination.

In rats, the oral dose of 2 mg/kg gives rise to blood levels approximately one quarter those obtained in dogs, the said levels disappearing within 12-13 hours; on increasing the dose the serum levels rise and are of the same order as those obtained after intravenous administration, even the highest level disappear after 24 hours and do not persist for 56 hours as in dogs (Furesz, 1970). In guinea-pigs also blood level disappears within 24 hours. Moreover, guinea-pigs require doses 4 to 5 times greater to reach the same serum levels as rats and mice. In mice, blood levels persist for over 40 hours.

The pattern in man is similar to that of rats and guinea-pigs and in any case quite different from that of dogs.

Repeated administration

During repeated administration of rifampicin, the serum concentrations and half-life decrease with time and
reach, at equilibrium, values which are generally lower than those observed after the first dose. This phenomenon has been studied experimentally with daily doses of 900, 600 and 300 mg given 12-hourly for 14 days (Acocella et al., 1971). This decrease is more marked for the 900 mg repeated dose and almost negligible for the 300 + 300 mg dose. A decrease in the half-life on repeated administration has also been observed by Boman (1974) after as few as 2 consecutive doses of 10 mg/kg of rifampicin. In this study, there was also a statistically significant regression between the changes in half-life for the first and the third consecutive dose and the initial half-life value, in the sense that the higher the initial value the more marked the decrease.

A portion of the rifampicin absorbed becomes bound to the plasma proteins and only unbound fraction displays antibacterial activity. Published data on the drugs protein binding vary greatly owing to differences in the methods of determination employed (Kenny and Strates, 1981).

Protein binding

From the data reported by Boman and Ringberger (1974), it is evident that the method employed was not without effect on the results obtained. However, it seems reasonable to estimate that the fraction of rifampicin bound to plasma proteins is around 80%.
Several investigators found that 30-41% of the protein-bound rifampicin is associated with the serum albumin fraction (E. Poli-Sandri and A. D'Alessio, 1971; Boman and Ringberger, 1974; Chojnowski and Gralewicz, 1976; Buchanan and Walt, 1977). The finding of no differences in total rifampicin protein binding in sera of albumin depleted or kwashiorkor (2.0-2.4 g albumin/dl serum) and normal (4.1 g albumin/dl serum) subjects suggest that gamma globulins rather than albumin may be the major serum binding site of rifampicin (Buchanan, 1977).

The protein fraction which acts as carrier has not been clarified. Curci et al. (1970) indicate albumin and Assandri (1977) albumin and the protein fractions I and III of Cohn (beta-lipoproteins, beta-lipid-poor euglobulin, ceruloplasmin, isoaglutinin, prothrombin). The binding is reversible and relatively weak. The affinity value suggests a percentage of free rifampicin of the order of 15 to 20%. Boman and Ringberger (1974) produced evidence indicating that rifampicin is bound to albumin (19 to 45%), gamma-globulin (5 to 20%) and fibrinogen (3 to 12%).

Diffusion/distribution in the body

Rifampicin diffuses rapidly from the plasma into the other body fluids and into the tissues and organs. Contributing to this rapid diffusion are its good liposolubility
and the fact that only about 25% is negatively ionized in blood which has a physiological pH. Two to five hours after oral administration, rifampicin can be detected in practically all body fluids, tissues and organs.

**Distribution in tissues**

The concentrations of rifampicin in various tissues have been evaluated on surgically removed samples after administration of doses of 150 mg of the antibiotic. Whenever possible, simultaneous blood samples were collected to establish the ratio of blood/tissue concentration at a given point in time after dosing. With doses of 450 mg and at a time interval of the order of 12 hours after antibiotic administration, bile, liver tissue, gall bladder wall, ureter and ovarian cyst wall and fluid were found to have a ratio lower than 1. A ratio of the order of 1 was found for such tissues as spleen, mesocolon cyst and fluid, appendix, skin and muscle, rib and mammary tumour tissue. A ratio greater than 1 was found for lung, stomach wall and tumour tissue, fat and breast milk.

In the parenchyma of the lung, rifampicin attains high concentrations, which on the whole are roughly on a par with those measured in the blood at the same time point (Furesz et al., 1967).
Rifampicin is also found in the bladder wall, prostate, seminal vesicles and in cerebrospinal fluid. Of special interest, in view of the most common clinical use of rifampicin, are the high concentrations in sputum and cavitary fluid of patients with tuberculosis (Binda et al., 1971).

The concentration in saliva may reach levels equivalent to roughly 20% of the serum concentration. Rifampicin crosses the blood-brain barrier. Where the meninges are normal, the cerebrospinal fluid concentration following oral dose of 600 mg range from 0 to 0.8 ug/ml (Pilheu et al., 1970). In patients with inflamed meninges values are higher (Barza, 1982), from 0.3 to 1.3 ug/ml or even more (Farr and Mandell, 1982).

**Metabolism**

The main derivative of rifampicin in man is desacetyl rifampicin, which is microbiologically active (although much less so than the parent drug) and represents the main fraction in bile. Desacytlation of rifampicin most probably takes place in the hepatocyte and results in a more polar compound which facilitates its excretion in bile.

Approximately 10% of the antibacterial activity found in human urine is represented by formyrlrifampicin, a derivative thought to form spontaneously in the urine, although
a metabolic origin cannot be excluded. Nakagawa and Sunahara (1975) have reported data indicating that a fraction of rifampicin could be conjugated with glucuronic acid in the liver, a finding which could explain the proliferation of the smooth endoplasmic reticulum of the hepatocyte associated with rifampicin administration.

In man, biotransformation of rifampicin molecule involves four chemical reactions: hydrolytic splitting off of the pyrazinylimido residue, oxidation of quinone, desacetylation at carbon atom 25 and demethylation at the piperazinylimido group.

The modern technique of high pressure liquid chromatography (HPLC) shows that the metabolites develop very rapidly, the concentration comes from a 'shoulder' about four hours after oral administration, a finding which is indicative of an enterohepatic circulation (Lecaillon et al., 1981).

Rifampicin

<table>
<thead>
<tr>
<th>Hydrolysis</th>
<th>Desacetylation</th>
<th>Oxidation</th>
<th>Demethylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-formyl-rifampicin</td>
<td>25-desacetylR</td>
<td>Rifampicin quinone</td>
<td>N-demethyl R</td>
</tr>
<tr>
<td>SV</td>
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</tbody>
</table>

Hydrolysis

<table>
<thead>
<tr>
<th>3-formyl-25-desacetyl rifampicin SV</th>
<th>Desacetyl rifampicin quinone</th>
</tr>
</thead>
</table>
Thus, not only the non-metabolized rifampicin-fraction but also at least to a certain extent, the metabolite desacetyl rifampicin is subjected to an entero-hepatic circulation, also supported by findings of Acocella et al. (1978).

**Biliary excretion of rifampicin in the rat and in the dog**

<table>
<thead>
<tr>
<th>Doses (mg/kg)</th>
<th>Hours after administration (ug/ml)</th>
<th>% administered dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2</td>
<td>2-4</td>
</tr>
<tr>
<td>Rat (n=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 i.v.</td>
<td>972</td>
<td>821</td>
</tr>
<tr>
<td>5 i.v.</td>
<td>333</td>
<td>233</td>
</tr>
<tr>
<td>2.5 i.v.</td>
<td>251</td>
<td>182</td>
</tr>
<tr>
<td>25 OS</td>
<td>461</td>
<td>350</td>
</tr>
<tr>
<td>Dog (n=2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 i.v.</td>
<td>174</td>
<td>104</td>
</tr>
</tbody>
</table>

In the rat, the biliary excretion is quite constant in the time interval examined, independent of the doses administered and the route of administration. Recovery of rifampicin from the bile during the 6 hours of observation is around 50% for i.v. and 16% for oral dose. In the dog, biliary excretion is constant in time, while the recovery is instead, about 10 times less than in rats (Furesz, 1970).
Nakagawa et al. (1970) observed that in the liver rifampicin becomes partly conjugated with glucuronic acid. This is consistent with the observation that the smooth endoplasmic reticulum of liver cells, in which the enzyme system responsible for breaking down the drug is located, proliferates during continuous administration of rifampicin (Jezequel et al., 1971; Pilheu et al., 1979).

The total amount of rifampicin excreted via liver, gall bladder and gut does not increase proportionately to the dose, because the ability of the liver to eliminate or store rifampicin reaches a saturation point (Acocella, 1978). In the bile, rifampicin rapidly appears in high concentration which persists even when the antibiotic can no longer be detected in the serum (Dettli, 1969). After 12 hours of oral administration of 150, 300 and 600 mg rifampicin, 29.6%, 20.4% and a mere 13.9%, respectively, of the dose could be detected in the bile (Miano and Peruzzi, 1969).

Brechbuhler et al. (1978), using a spectrophotometric method of determination, found that the percentage of apparent rifampicin (i.e. unchanged plus 25-desacetyl rifampicin) measurable in urine steadily increases as the dose is raised from 150 to 900 mg. This phenomenon is in all probability due to a dose-dependent first-pass effect,
in which the limited capacity of the microsomal liver protein to bind rifampicin plays a role.

**Biliary excretion**

Rifampicin is present in bile during the first hour following oral administration with concentrations increasing and reaching a plateau 4 to 5 hours after administration. The excretory capacity of the liver for rifampicin is saturated at doses of 300 to 450 mg. Indeed, a 4-fold increase in dose (from 150 to 600 mg) does not result in a corresponding increase in bile concentrations (Acocella et al., 1967).

**Urinary excretion**

**Single dose administration**

After administration of therapeutic doses, rifampicin is rapidly excreted in urine; the pattern of the urine concentrations following rather closely that of the serum levels (Miano and Peruzzi, 1969). It has been noted that for doses ranging from 150 to 600 mg, peak urine levels are reached at 6 hours with concentrations ranging from 100 to 450 μg/ml. In terms of recovery in 12 hours, Miano and Peruzzi (1969) report values of the order of 13% for the 150 mg dose, 17% for the 300 mg dose and 24% for the 600 mg dose.
Repeated administration

Rifampicin levels in urine follow the time course pattern of the serum concentrations. Therefore, as a result of the decrease in the serum concentrations on repeated administration, the urine levels and recoveries also decrease with time over the first days of treatment (Gomi et al., 1969). As expected, the decrease in urine concentration is reflected in the recovery data. With daily doses ranging from 1200 mg, there was a decreased recovery of the order of $\frac{1}{2}$ to $\frac{1}{7}$ at day 9 to 16 of treatment in comparison with day 1 data.

Renal clearance

The mean value, expressed in ml/min/1.73 m$^2$ body surface in normal subjects, was $30.2 \pm 3.6$ significantly ($P \leq 0.01$) more rapid than in 2 groups of patients with reduced renal and liver function. According to Reubi et al. (1970), the renal clearance of rifampicin corresponds, on average, to 12.2% of the glomerular filtration rate (range 2.2 to 24.2%) and 2.4% of para-aminohippuric acid clearance (range 0.5 to 5.8%).
Pharmacokinetics in children
Infants and small children

Following a single dose of 10 or 20 mg rifampicin per kg body weight, the drug's half-life in infants and small children aged 1½-14 months averaged 2.6-2.9 hours (Krauer, 1969). As compared with adults, there is thus no appreciable difference in the elimination rate; the tendency towards a somewhat longer half-life in the youngest children in this age group (i.e. those aged less than three months) was not significant.

However, the peak concentrations of rifampicin in infants - with the exception of newborn infants - attained only about one-third of the adult levels, although the dosages given were proportionally equivalent to the adult dosages (Acocella et al., 1970). Since the time course of rifampicin concentrations in the serum and urine is similar for infants and adults, this difference in the peak concentrations is presumably due to differences in the distribution volume. The distribution volume in this group (1½-14 months) is more than double that in adults, although extremely large individual variations may be observed (Krauer, 1969). Because in response to oral doses they show relatively low serum concentrations as compared with adults, it is recommended that the daily dosage for infants and small children should be set at 15 mg/kg.
body weight, to be taken on an empty stomach in the morning before breakfast. It would be even better to calculate the dosage according to body surface area (Acocella et al., 1970; Simon, 1975). During treatment of relatively prolonged duration, the same phenomenon is observed in children as in adults i.e. the peak serum concentrations decrease with time (Kofman et al., 1969).

Newborn babies

In the newborn, peak concentrations are reached considerably later than in adults and older infants. After only 2-3 days' treatment, however, the shape of the concentration curve changes, relatively high blood levels indicating some degree of accumulation - now already being observed 2-4 hours after administration of the drug (Acocella et al., 1970; Acocella, 1978). This accumulation, which is not observed in older infants and adults (Simon, 1975) and which is a sign that the liver enzyme system is not yet fully developed, must be taken into account when prescribing. In other words, premature and newborn babies should be given rifampicin only in an emergency and in a dosage never exceeding 10 mg/kg. A newborn baby's kidneys are not capable of compensating for the inadequate drug-eliminating capacity of its liver.
Microbiology

Mechanism of action

By forming complexes with ribonucleic acid (RNA) polymerase, rifampicin inhibits RNA synthesis, thereby blocking bacterial protein synthesis. Rifampicin thus interferes at a very early stage in the process by which information stored in deoxyribonucleic acid (DNA) is transformed, via transcription and translation, into proteins, thereby becoming functionally active in the cell (Binda et al., 1971).

Since each molecule of rifampicin binds to one RNA polymerase molecule, with which it forms a stable 1:1 complex, extremely low concentrations of this antibiotic (≤ 0.01 ug/ml) are sufficient to inhibit the bacterial enzyme (Wehrli and Staehelin, 1974). In contrast to bacterial RNA polymerase, mammalian RNA polymerase is inhibited by rifampicin only at concentrations 100-10,000 times higher. This specific affinity for the RNA-polymerase of bacteria, and in particular for the RNA polymerase of M. tuberculosis, distinguishes rifampicin from various cytotoxic antibiotics (e.g. actinomycin and mitomycin) which likewise interfere with RNA synthesis but become bound to DNA as well.
The binding site of rifampicin is the beta-subunit of RNA polymerase, and the drug thus prevents the initiation of new RNA chains; in mycobacteria, on the other hand, rifampicin exerts no influence on the elongation of chains already undergoing synthesis (Doub, 1979; Winder, 1982).

Investigations on the respective structure/activity relationships have revealed that the macrocyclic ring has an important bearing on the binding to RNA polymerase, while the aromatic nucleus plays a large part in determining penetration into the bacteria (Winder, 1982).

The fact that rifampicin is unrelated in its chemical structure to any of the commonly used antibiotics is reflected in the absence of cross-resistance (except with other rifamycins).

**Structure of action**

Rifampicin is highly active against *M. tuberculosis*, *M. bovis*, *M. africanum* certain atypical mycobacteria, as well as against many gram-positive bacterial species, particularly staphylococci (including penicillinase-producing strains and penicillin-resistant staphylococci) and also against various gram-negative organisms, notably *Neisseria gonorhoea*, *Escherichia coli* and *Proteus*. 
In vitro activity

Inhibitory concentrations

The minimal inhibitory concentrations (MICs) of rifampicin generally lie within the range of 0.002-0.5 ug/ml for gram-positive organisms and about (0.02)-1 to 10 ug/ml for gram-negative organisms (Helwigg, 1976).

Rifampicin, in common with isoniazid, has the lowest MIC for tubercle bacilli. Otten et al. (1975) report ranges of (0.005-0.05) - 0.5(-5) ug/ml in semisynthetic culture media and of (0.5-)1-5-10(20) ug/ml for egg medium. How much the MIC values depend on the composition of the culture medium is illustrated by the findings of Pallanza et al. (1967) and Hobby and Lenert (1968), who, having recorded MICs of 0.04 ug/ml for the H37Rv strain and 1 ug/ml for clinical isolates when they employed either Dubos medium or modifications of it, observed that these values fell to 0.005 ug/ml and 0.02-0.08 ug/ml, respectively, after addition of Tween.

Using Proskauer-Beck medium, Pallanza et al. (1967) found that the MICs for rifampicin and isoniazid, at 0.05 ug/ml each, were about ten times lower than those for streptomycin and PAS (0.5 and 0.2 ug/ml, respectively).
With proliferating organisms, the bactericidal concentration of rifampicin for M. tuberculosis is close to the MIC. After six days' incubation with 0.5 μg/ml in Dubos medium, only 5.6% of the bacilli survived. On Lowenstein-Jensen medium, total inhibition of the H37Rv strain was observed with 5 μg/ml (Pallanza et al., 1967). Otten et al. (1975) reported bactericidal concentrations of between 0.05 and 30 μg/ml, depending on the medium, on the incubation time, and on the size of the inoculum.

The effect of combining rifampicin with various other antituberculous drugs in vitro can be seen. With rifampicin alone the bacterial count was reduced by about two powers of ten in 20 days, whereas the combinations lowered the bacterial count by approximately two further powers of ten. Worthy of note, however, is the fact that, at the concentrations employed, it was only after exposure to rifampicin that those organisms which could still be counted no longer proved cultivable, i.e., had lost their ability to multiply in culture.

In the light of the aforementioned figure, it follows that serum concentrations 50 to 100 times greater than the in vitro MIC can be achieved with appropriate dosages of rifampicin.
Autoinduction by rifampicin

Studies with $^{14}$C-labelled rifampicin have shown binding of the drug to hepatic microsomes (Riess et al., 1969). This, in addition to its highly lipophilic properties (Curci et al., 1970), may account for the proliferation of smooth endoplasmatic reticulum observed in human and guinea-pig hepatocytes (Jezequel et al., 1971; Hakim et al., 1973). Furthermore, in the homogenates of needle-biopsy specimens of human liver, the content of cytochrome P-450 and the N-demethylation of aminopyrine was enhanced 3 to 5-fold in patients with tuberculosis receiving rifampicin (Schoene et al., 1973). Urinary D-glucaric acid excretion, a possible indicator of hepatic microsomal activity in man (Hunter et al., 1973), increased significantly during antituberculous therapy with rifampicin (Edwards et al., 1974; Breimer et al., 1976). When the drug was given during a 7 days period, a reduction of its own half-life occurred (Acocella et al., 1972). In patients on long-term treatment, Nitti et al. (1972) also observed a significant decrease in the half-life of rifampicin.

Besides stimulating its own metabolism, rifampicin influences the elimination of several other drugs.
Experimental induction of drug metabolism

Rifampicin is a potent inducer of drug metabolism in humans, which may give rise to drug interactions of practical relevance. These observations have been confirmed in an experimental investigation by Zilly et al. (1975a) on the influence of rifampicin on the pharmacokinetics of hexobarbitone and tolbutamide, both of which are almost completely metabolized in the liver. Before and after 8 days of rifampicin treatment (1.2 g daily orally) healthy volunteers received hexobarbitone (7.32 mg/kg) and tolbutamide (20 mg/kg) by i.v. infusion on 2 consecutive days. The average elimination half-life of hexobarbitone had decreased from 325 to 122 min and of tolbutamide from 148 to 183 min following rifampicin treatment. The metabolic clearance of hexobarbitone increased about 3-fold and that of tolbutamide more than 2-fold (Zilly et al., 1975a). Changes in the distribution behaviour of the two drugs were not observed. As with digoxin, no influence on protein binding of tolbutamide, which is also largely bound to plasma proteins (Held et al., 1973) occurred. A similar rifampicin-induced reduction of the half-life of tolbutamide had previously been reported.

The shortened half-life of both hexobarbitone and tolbutamide is the result of an increased metabolic
clearance, indicating an extensive stimulation of hepatic microsomal drug metabolizing enzymes by rifampicin. In a following study, it was found that within 14 days the clearance of hexobarbitone had returned to normal values (Breimer et al., 1976). As has been described in animal experiments after treatment with the enzyme inducer phenobarbitone (Conney, 1967), an increase in liver volume by about 15% was observed in humans after about 8 days treatment with rifampicin (Zilly et al., 1975); liver volume returning to pre-treatment values within a fortnight. It is noteworthy that this effect was not accompanied by signs of liver cell injury, as judged by conventional liver function tests.

Unexpected results were obtained when antipyrine was used as the test drug (Breimer et al., 1976). In contrast to hexobarbitone, plasma concentrations of antipyrine were comparable before and after 8 days treatment with rifampicin. The average half-life of antipyrine was 6.9 hours before and 7.2 hours after rifampicin treatment. The results of an experiment with antipyrine in comparison with those obtained for hexobarbitone, tolbutamide and digitoxin, suggest that rifampicin does not enhance the elimination rate of antipyrine, which is in contrast to the effect of phenobarbitone in this respect (Vesell and Page, 1969). Therefore, it
appears that rifampicin is a selective inducer of oxidative
drug metabolism, which implies that not every compound
metabolized by oxidation will be affected. It is possible
that multiple forms of cytochrome P-450 might be differenti­
tially enhanced by a particular inducer such as rifampicin
(Lu et al., 1976).

Effect of enzyme inducing drugs on rifampicin

Since rifampicin has also been shown to enhance its
own rate of biotransformation, the question has arisen
whether it is also affected by well known inducers of drug
metabolism such as phenobarbitone and ethanol. De Rautlin
De la Roy et al. (1971) showed that phenobarbitone treatment
lowered the plasma concentration of rifampicin, while
Nitti et al. (1973) observed a decrease of rifampicin
half-life. However, the results of similar studies by
Acocella et al. (1974) have indicated that although there is
a tendency for phenobarbitone to shorten the rifampicin
half-life slightly, the phenomenon is of little clinical
relevance. With respect to ethanol, no clinical data are
available, but experiments in rats chronically treated with
ethanol have indicated that the rate of biotransformation
of rifampicin is enhanced under these circumstances
(Grassi et al., 1972; Grassi and Grassi, 1975). However,
it would be premature to extrapolate these data to the
clinical situation.
**Other possible interactions**

It is interesting to note that contradictory effects of rifampicin on hepatic drug metabolism in animals have been observed. On acute administration, inhibition of hexobarbitone metabolism and of other metabolic pathways occurred in mice and rats, whereas daily administration for 6 days led to an increase in the activity of several microsomal enzyme reactions (Pessayre and Mazel, 1976). However, this only occurred in mice, whereas in rats a specific induction of NADPH-cytochrome C reductase and ethylmorphine demethylase was found, without influence on cytochrome P-450 content, demethylation of aminopyrine and p-nitro-anisole, oxidation of aniline and glucuronidation of p-nitrophenol and chloramphenicol (Otani and Remmer, 1975). The acute inhibitory effect of rifampicin may be due either to a direct effect at the enzyme level, or to interference with hepatic uptake of substrates. Kenwright and Levi (1974) have shown that rifampicin does interfere with hepatic transport mechanisms. Thus, rifampicin and probenecid were shown to have similar effects in depressing the hepatic uptake of bromsulphthalein (BSP) and bilirubin in rats.

In man, during infusion of rifampicin a rise in BSP retention, a decrease in indocyanine green (ICG) plasma disappearance rate and an increase in bilirubin levels were observed (Acocella et al., 1965). On the other hand,
when cholecystographic contrast media and rifampicin were given simultaneously, high plasma levels of rifampicin resulted (Curci et al., 1969). In addition, Kenwright and Levi (1973) found that peak serum levels of rifampicin after a 300 mg dose were raised by 86% following oral probenecid administration. Mean serum concentrations increased by approximately 100% at 4, 6 and 9 hours. The authors initially suggested that this finding may have important therapeutic implications because the use of rifampicin is often limited by cost. However, subsequent studies showed little justification for use of probenecid to reduce the usual dosage of rifampicin (Fallon et al., 1975; Allen et al., 1975).

Although it is clear that rifampicin interactions may also occur at the level of hepatic drug uptake or at the level of biliary excretion (Acocella and Billing, 1965), the clinical relevance of these mechanisms with respect to interactions with other drugs would be premature to extrapolate these data to the clinical situation.

Patients with impaired liver function

In patients with impaired liver function, serum concentrations of rifampicin are higher than those obtained with corresponding dose levels in normal subjects. Furthermore, the biological half-life of the antibiotic is significantly
increased. Capelle et al. (1972) administered a daily 600 mg dose of rifampicin for 17 consecutive days to 4 patients with impaired liver function (cirrhosis) and normal controls. In contrast with the controls, the serum concentrations (at 8 hours after administration) were very constant; around values of 8 ug/ml. In the cirrhotic patients, the mean half-life of rifampicin was found to be $5.42 \pm 0.55$ hours, significantly longer ($P < 0.01$) than that in the controls ($2.80 \pm 0.22$ hours). In both groups, the half-life decreased notably during the first week of treatment.

The nature of the liver disease, or whether the condition is acute or chronic, does not seem to affect the general finding of decreased clearance in liver disease. Thus, serum rifampicin concentrations are higher than those in controls whether the liver impairment is chronic hepatitis, acute viral hepatitis or alcoholic cirrhosis. The time course of the serum concentrations is clearly consistent with a prolongation of the half-life.

Patients with impaired kidney function

Renal clearance of rifampicin is reduced in patients with impaired renal function. In a comparative study, De Gregorio et al. (1970) found clearance to be $30.2 \pm 3.6$ ml/min/1.73 m$^2$ body surface in a group of healthy controls.
compared with a significantly (P < 0.01) reduced rate of $12.3 \pm 5.6$ ml/min/1.73 $m^2$ in a group of patients with impaired kidney function. Similar studies were carried out by Reubi et al. (1970). As a working hypothesis, these authors assumed a degree of binding of rifampicin to serum protein of the order of 75%.

If an active transport mechanism for renal excretion is not involved, the clearance should not exceed 25% of the glomerular filtration rate (GFR). The results showed lower than expected values: the clearance of rifampicin was in fact found to correspond to 12.2% of the GFR (range 2.2 to 24.2%) and to 2.4% of para-aminohippuric acid clearance (range 0.5 to 5.8%). On the basis of these findings, it was concluded that rifampicin could be administered at normal therapeutic doses to patients with severe impairment of renal function.