

TRADITIONAL CHINESE HERBAL ANTHELMINTICS :

THEIR HISTORY

AND

LABORATORY EVALUATION

by

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Abstract

Traditional Chinese herbal anthelmintics (abbreviated as TCHAs) have been used for over 2000 years. Many commonly used TCHAs have not been investigated scientifically.

TCHAs were evaluated through a historical review of Medical Parasitology in China, an enumeration and tabulation of 121 anthelmintic prescriptions and their uses, and a review of the small number of previous experimental studies. This led to the subsequent laboratory experimentation to assess their chemotherapeutic value.

The laboratory evaluation included in vivo studies using Nippostrongylus brasiliensis and Hymenolepis diminuta, and in vitro study on Ascaris lumbricoides. The herbs tested were Areca catechu, Cucurbita pepo, Melia azedarach (seed and root), Mylitta lapidescens, Quisqualis indica, and Ulmus macrocarpa.

The laboratory results showed that the therapeutic value of these TCHAs in the rat-parasite model compared very poorly with western anthelmintics. It is argued that the effect of medicine is complex and depends on many socio-cultural factors. These, it is proposed play essential roles in their continuous widespread use.

Abrégé

Les plantes Chinoises traditionnelles anthelmintiques (PCTA) sont en usages depuis plus de 2000 ans. Cependant plusieurs de ces plantes n'ont pas été vérifiées tant qu'à leurs valeurs thérapeutiques réelles.

Ces plantes furent évaluées à la fois dans une perspective historique de la médecine Chinoise traitant des parasites, une énumération et tabulation de plus de 121 prescriptions et usages d'anthelminthes, et une revision de quelques études expérimentales. Subséquament ceci a mené vers des expérimentations afin de vérifier leurs valeurs chemotherapeutiques.

Ces expérimentations comprennent des études in vivo utilisant Nippostrongylus brasiliensis et Hymenolepis diminuta, ainsi que des manipulations in vitro sur Ascaris lumbricoides. Les plantes vérifiées furent Areca catechu, Cucurbita pepo, Melia azedarach, Mylitta lapidescens, Quisqualis indica et Ulmus macrocarpa.

Les résultats démontrent que la valeur thérapeutique des PCTA chez le modèle rat-parasite, se compare désavantageusement avec les anthelminthes occidentaux. Cependant on peut noter que les effets de la médecine sont complexes et peuvent dépendre de plusieurs facteurs socio-culturels.

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INTRODUCTION

The selection of traditional Chinese herbal anthelmintics as a subject of study.

Various medical systems have evolved independently. Each system has its own types of healers, its varied pharmacopoeias and therapeutic paradigms, and its own validity within the framework and constraints of its particular cultural boundaries and stage of development (Weidman, 1979). A biotraditional (Jelliffe and Jelliffe, 1977) system which developed quite distinctly from those of Europe is traditional Chinese medicine (Needham, 1972).

Chinese medicine probably started about three thousand years ago (Kao, 1973). Keele postulated that probably the first civilized people to liberate themselves from the "purely magico-religious concepts of disease were the ancient Chinese" (cited by Needham and Lu, 1969). The folk medicine of the Chinese evolved into systematic traditional Chinese medicine (Croizier, 1968; Kao, 1973), which has been described as a well-organized system of knowledge (Li, 1974; 1975), and having a rationality of its own (Croizier, 1968; Dubos, 1979; Porkert, 1976a). It is based on a holistic concept, integrating the body, the mind, and the total environment.

The Chinese had always emphasised hygiene and preventive medicine (Bridgman, 1974). Fundamental ideas on preventive medicine appeared as early as the seventh or eighth century B.C. in the I-Ching. It was stressed throughout the Huang Ti Nei Ching, Su Wen, The Yellow Emperor's Classic of Internal Medicine, that the responsibility of the physician was not only to attend to the sick but also to maintain the health of the healthy (Needham, 1970). Since the social, cultural, and

hygienic conditions of today differ so drastically from that of China, a thousand years ago, it is difficult to evaluate the efficacy of prophylactic practices used a thousand years ago.

In the oldest Chinese medical classic, The Yellow Emperor's Classic of Internal Medicine, it was stated that there were five methods of treatment. The first method of treatment was the cure of the spirit (Veith, 1973). This seems similar to the principles of logotherapy, pioneered by Viktor Frankel (1976). The nourishment of the body, which is reminiscent of present-day dietetics and nutritional science (Williams, Heffley, Yew, and Bode, 1979) is the second method. Chemotherapy constitutes the third method of treatment. The fourth method instructs the physician on the treatment of disorders of the digestive system and the visceral organs by insisting on the evacuation of the intestinal tract and renal excretion. This could be identified with the principles of the methods used today for treating chemical intoxication (Wintrobe, Thorn, Adams, Braunwald, Isselbacher, and Petersdorf, 1974). The fifth method involves the application of acupuncture and moxibustion. The method most appropriate for laboratory evaluation is the third, namely, the effect of chemotherapy.

The Chinese pharmacopoeias contain numerous descriptions of simple medicines of vegetable, animal, and mineral origin (Cooper and Sivin, 1973). Ephedra sinica has been used by the Chinese as a diuretic and a treatment for asthma for a long time (Pai, 1972). Ephedrine, an active compound extracted from it, is used for the treatment of asthma and hay fever today (Chen, 1972; Wintrobe et al., 1974). For at least a thousand years, Dichroa febrifuga has been known to the Chinese as an antimalarial and an antiseptic (Huard and Wong, 1968;

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Jang, Fu, Wong, Huang, Lu, and Chou, 1946; Palos, 1972). Leonurus was first used as a diuretic but later its antifertility properties were observed (Huard and Wong, 1968; Yun, Hu, Lau, Che, Yeung, Cheng, and Hwang, 1976). The herb known for improving metabolism is Glycyrrhiza uralensis (Palos, 1972; Read, 1925-1926). Panax ginseng is noted for its restorative properties (Hu, 1977; Palos, 1972). The traditional Chinese pharmacopoeias are thus a source of herbal remedies. Increasing attention is being paid to herbal treatment (Geissman, 1964; Halberstein and Saunders, 1978) and ethnobotany (Schultes, 1960). The armamentarium of written pharmacology of today basically consists of traditional herbal remedies which have been investigated (Burgen and Mitchell, 1978; Dunn, 1976; Farnsworth and Bingell, 1977; Kreig, 1964; Schultes, 1972), and whose active ingredients have been purified and their synthetic analogs have been made. Furthermore, drugs of plant origin usually have milder adverse effects than synthetic drugs (Petkov and Manolov, 1978; Stuart, 1975), or minerals and drugs of animal origin, which are often prescribed in Chinese traditional medicine. The widespread use of Western chemotherapeutic drugs may result in this potential source being overlooked and lost. The Chinese herbal pharmacopoeias are voluminous and the immediate problem is to make suitable selections.

Most of the people infected with parasitic diseases live in the less-developed countries. The costs involved in screening thousands of drugs and developing those that show some promise are enormous (Standen, 1976). Effective anthelmintics for some parasitic diseases exist but many of the best are too expensive to be economical (Albert, 1975). Profit-dependent and profit-motivated pharmaceutical companies

cannot find adequate markets which can afford the new effective human antiparasitic drugs (Duke, 1978). The absence of patent laws in most less-developed countries and the increasingly stringent criteria set by WHO and FDA (Bryceson, 1976; Georgi, 1974) further discourage innovation. It is, thus, not surprising that a review of the progress in chemotherapy charts only a slow expansion in parasite chemotherapy (Bryceson, 1976; Chain, 1976; Gadebusch, 1976). Antiparasitic drugs are likely to continue to play an important role in the integrated control strategy of parasites, involving control, vaccination, and cultural practices (Campbell, 1977). Since there are so many people suffering from helminthiases, and the progress in parasite chemotherapy is slow, there is dire need for inexpensive antiparasitic agents.

The paucity of information on parasite and host biochemistry is not conducive to developing antiparasitic drugs by the rational approach. Besides, only 5% of the 600,000 plant species existing on the earth have been specifically screened and investigated chemically or pharmacologically (Wagner and Wolff, 1977). The screening of traditional Chinese herbal anthelmintics (abbreviated as TCHAs) could perhaps fill this need.

Several characteristics of the traditional Chinese medical system can be identified in traditional Chinese herbal anthelmintic prescriptions. These prescriptions are holistic in principle. They consist of active anthelmintic ingredients, detoxifying herbs, and herbs which improve the "general health" and metabolism of the patient. These prescriptions have remained unchanged for over two thousand years. They did not disappear with the advent of Western medicine. TCHAs are not part of folk medicine since they are well-documented in Chinese

medical texts. They are not parochial since a list of herbs could be recognized as anthelmintic by Chinese pharmacists from Canada, China, Hong Kong, or Malaysia.

The hypotheses which are postulated to explain why traditional Chinese medicine did not undergo scientific revolutions and adhered to its traditional principles until the late nineteenth century, and why, even when Western medicine was introduced and accepted, traditional Chinese medicine did not disappear, could also be applied to explain the longevity of TCHAs.

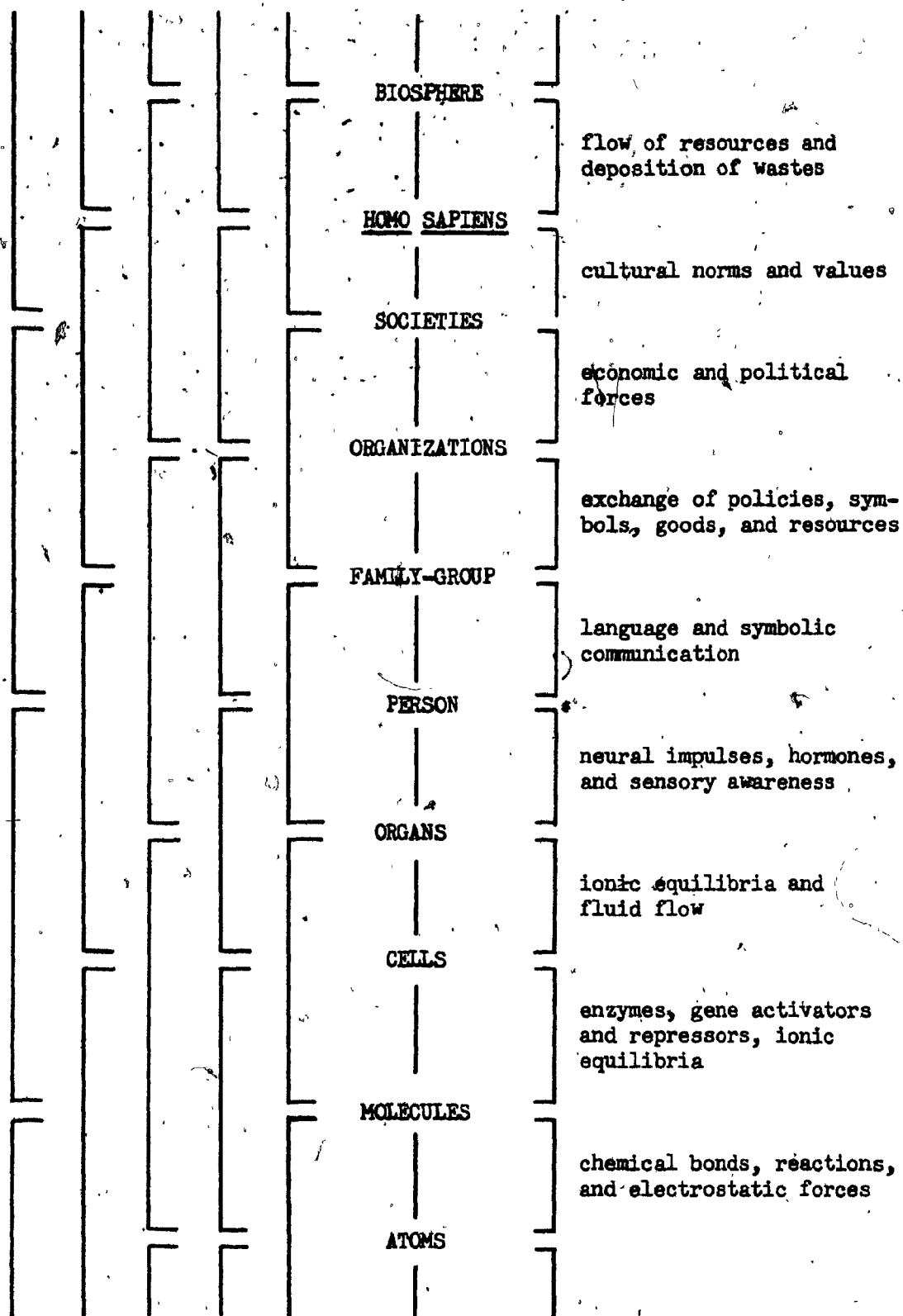
Modern science, which is responsible for the reductionistic biomedical model presently characterizing modern scientific medicine, did not develop in China. Anthropologists (Farb, 1978; Harris, 1971) and historians (Needham, 1970; Needham, 1972) attributed it to the Chinese political-socioeconomic system, which was characterized by its "hydraulic civilisation" (term coined by Karl Wittfogel) and "oriental despotism". The absence of democracy and capitalism which provided the financial support for scientific research in the West from the late eighteenth century onwards, and the non-existence of geometrical and mathematical thought were some of the other contributing factors postulated (Needham, 1972). Needham expounded on this question comprehensively in The Grand Titration: Science and Society in East and West.

Traditional Chinese medicine, including TCHAs, did not disappear with the advent of Western science and medicine. It seems paradoxical that twentieth century intellectuals committed to science and modernity should have continued to support traditional Chinese medicine (Croizier, 1968). One of the possible explanations is that traditional Chinese medicine has therapeutic value. It is very expensive to provide

Western medical care to eight hundred million people. Utilising traditional Chinese medicine and the already existing pool of traditional medical practitioners would be less expensive and perhaps more effective since the Chinese are more willing to accept Chinese medicine than Western medicine. Science is "oecumenical" but medicine still "remains part science and part social science, or perhaps part cultural" (Croizier, 1968). Medicine is thus, adopted as a symbol of its cultural nationalism. Besides, it was possible for the Communist leadership to accept and identify with the old system because medicine was regarded by the Confucian literati as a tradesman's calling (Croizier, 1972). By 1954, so much of the old culture and its societal matrix were destroyed that the Communists, confident of their power, could revive traditional Chinese medicine as part of the Chinese national heritage. The hypotheses postulated to rationalize why modern science did not develop in China and why traditional Chinese medicine remained popular even with the introduction of Western medicine, illustrate the complex interaction of ideological factors with cultural, geographical, political, social, and economic conditions, and structures involved in Chinese medicine (Croizier, 1968; Dull, 1976; Huard and Wong, 1968; Needham, 1972).

The mechanism of this complex interaction from the individual level to the societal and environmental level could be hypothetically explained by this model of information (Figure 1). A living organism is a hierarchy of open systems (Bertalanffy, 1968), each of which is continuously exchanging matter, energy, and information with the environment (Brody and Sobel, 1979). This series of complex interactions between a living organism and its environment manifests itself

Figure 1: Information flow in the hierarchy
(from Brody, H. and D.S. Sobel. 1979.
In: Ways of Health, ed. D.S. Sobel,
pp. 87-104. New York: Harcourt Brace Jovano-
vich).



in the form of a thermodynamic struggle (Dubos, 1977). A living system responds by adapting its physiological and psychological processes accordingly to adjust to environmental conditions. "Health" thus can be broadly defined as the "ability of a system (for example, cell, organism, family, society) to respond adaptively to a wide variety of environmental challenges (for example, physical, chemical, infectious, psychological, and social)" (Brody and Sobel, 1979). Health is noted here not as the mere absence of signs and symptoms of disease but the ability to respond. It is not restricted to biological well-being but also includes the environmental, sociocultural, and behavioural determinants of health which exist at different levels of integration. It views health as a dynamic process. It has, however, not overlooked the fact that the finer details of the criteria of health differ with the environmental conditions and with the norms and history of the social group (Dubos, 1971). As an instinctive defence mechanism, human beings in illness have looked for plants, animal parts, or minerals which could relieve or cure them of the illness. Thus, early pharmacological therapy and the beginnings of medicine were born of instinct (Sigerist, 1955) but later channelled by cultural and ecological restraints and opportunities (Harris, 1977). It evidently follows that there is a close bond linking health/disease, human nature, and medicine (Landy, 1977). This is illustrated in a review of the history of Chinese medicine. Since the review revealed a strong influence of culture, politics, and socio-economic factors on the development of Chinese medicine, a laboratory evaluation of TCHAs in rats would eliminate the psychological factor and concentrate on the chemotherapeutic effects alone.

Thesis Outline.

Three approaches to the study of TCHAs are adopted here:

- 1) A survey of the literature available,
- 2) A laboratory investigation of their efficacies in laboratory models,
- 3) A field study of the use of TCHAs.

The first two approaches form the main thesis. Since a field study of the use of TCHAs is not possible, the situation is rectified by collecting published literature.

This dissertation consists of four parts. Part I focuses on the theoretical orientations in Chinese medical parasitology while Parts II and III concentrate on the laboratory evaluation of TCHAs. Part IV is a synthesis of Parts I and III.

Part I is further divided into 5 sections. The first is a review of the history of Chinese medicine, with emphasis on the development of parasitology. It is not possible to isolate the study of traditional Chinese anthelmintics from the history of Chinese medicine. A review of the history of China is a prerequisite to any analysis of its system of medicine (Bridgman, 1974). The hypotheses postulated on the origin of parasites are discussed in Section B as a knowledge of them is essential for the orientation of the approach to the study. Sections A and B are theoretical attempts to elucidate the rationale of Chinese anthelmintic treatments. Selected traditional Chinese herbal anthelmintic prescriptions are listed in Section C. They form the basis of the selection of anthelmintics for a laboratory evaluation. Section D centres on the current studies done on the efficacy of traditional Chinese herbal medicine. An attempt is made to evaluate the efficacy

of TCHAs from a theoretical viewpoint in Section E.

The laboratory evaluation consists of two parts. Part II is a literature review of the techniques and the methodology used. Section A defines the Scientific Method. In Section B, some basic principles of pharmacology are applied to the laboratory evaluation since it is essentially a chemotherapeutic study. This is incorporated with a brief note on laboratory host-parasite models, which includes the problems encountered, choice, and criteria used in the selection of laboratory host-parasite models. A résumé of in vivo and in vitro experiments in parasitology forms Section C. It is followed by a review of literature on pyrantel pamoate, niclosamide, piperazine, and arecoline as these are the Western reference drugs used. The description of the herbal plants used are given in Section E.

Part III consists of the experimental materials and methods, results, and discussion of the laboratory evaluation.

Part IV ventures to integrate the theoretical and laboratory evaluations of traditional Chinese herbal anthelmintics.

The footnotes are collected at the end of each section.

PART I

Section A : History of Chinese medicine, with emphasis on medical parasitology.

A literature search revealed the paucity of published work in English on the historical aspects of the development of parasitology in China. A recent paper (Chan and Croll, 1979) attempts to rectify this situation since most published works allocate only a small section to parasitology and the information is dispersed. Chu and Ch'iang (1931), Chao (1940), Hoeppli and Ch'iang (1938; 1940; 1942; 1950; 1950-1; 1953), Hoeppli and Tang (1942), and Hoeppli (1950-1; 1952; 1954a; 1954b; 1954c; 1955; 1959) were perhaps the few collaborators responsible for translating the parasitological works. This section updates and augments the recent attempt to compile the fragmentary information on medical parasitology in China.

The Chinese Materia Medica is a heterogeneous, comprehensive, and highly rational body of theory and practice bonded by the most fundamental concepts of classic natural philosophy which have made adaptations to accommodate the phenomena of health and disease (Cooper and Sivin, 1973). Chinese medicine is interested in function as opposed to substratum (Porkert, 1976b), resulting in the inductive and synthetic modes of cognition being utilised, instead of the causal and analytical; adopted by Western science (Porkert, 1974). Consequently, traditional Chinese medicine is based on a holistic concept integrating the body, the mind, and the total environment (Agren, 1976; Cooper and Sivin, 1973; Dubos, 1979). The traditional Chinese medicine approach to health is consistent and coherent with the "General System Theory" postulated by Bertalanffy (1968) which has pervaded to all fields of

science including medicine (Brody and Sobel, 1979).

The historical development of Chinese medicine could be arbitrarily divided into three stages. The early development of Chinese medicine spanned from antiquity to 1000 B.C. followed by a period of systematic traditional Chinese medicine which lasted for about 3000 years from approximately 1000 B.C. to 1949. The new period of Chinese medicine started in about 1949 and continues into the present (Kao, 1973).

It is difficult to separate facts from legends during this period of early development of Chinese medicine. The three legendary figures mentioned below played important roles in shaping the thoughts of the Chinese.

Fu Hsi was thought to have lived around 4000 B.C. (Kao, 1973). The invention of the Pa-Kua or the system of octagrams was attributed to him. The I-Ching or the Book of Changes was influenced by the discovery of the Pa-Kua. The I-Ching had greatly influenced Chinese thought and philosophy besides being of fundamental importance to the art of healing. The concept of Yin = negative (for dark, cold, feminine) and Yang = positive (for light, warm, masculine) first appeared in the I-Ching (Palos, 1972). The balance of Yin and Yang is often used to describe disease. Fu Hsi represents the medical approach through a cosmic philosophy which believes that the universe is at the same time an unity and yet made of dual opposing forces like the Yin-Yang concept. Although Fu Hsi is closely associated with the metaphysical side of medicine and honoured as the first to understand the pulse, he is also regarded as the "prototype of magic and superstition, with its amulets and talismans and its search for the elixir of life"

(Hume, 1940).

Shen Nung, the "Divine Husbandman", probably lived around 3000 B.C. (Kao, 1973). He is also known as the Father of Chinese medicine. He experimented on the medicinal properties and uses of plants by chewing the plants himself (Wallnofer and von Rottauscher, 1964). The Pharmacopoeia of Shen Nung (Shen Nung Pen Tsao Ching) contained the names and descriptions of 365 different medicaments, together with some guiding principles for their use (Huard and Wong, 1968). He represents the approach through medication. He is revered as a symbol of the belief in empirical knowledge which has been "so characteristic an aspect of China's mental equipment through the ages" (Hume, 1940). To the Chinese, he typifies that "almost universal acceptance of the idea that medicaments are potent not only because they produce measurable pharmacological effects but because they correspond, in some mysterious way, to the patient's affected organs and possess, some magic power to drive harmful spirits away" (Hume, 1940).

Huang Ti or the "Yellow Emperor" is believed to have lived around 2700 B.C. (Kao, 1973). According to legends, he was responsible for the influential Huang Ti Nei Ching or The Yellow Emperor's Classic of Internal Medicine. Ilza Veith translated part of it into English in 1949 and later revised it in 1965. She wrote an interesting explanation of the age and authorship of the Nei Ching. Sections from the Nei Ching deal with helminthic infections, their causes, diagnoses, and treatment. It is stated in this classic: "In the human body, there are 80 000 corpse worms without which the human body cannot exist" (Hoeppli and Ch'iang, 1938) and "that eating and drinking beyond one's capacity will injure one's stomach and intestines. Indulgence in

eating fish, meat, wine, raw and cold food, fats and sweets also produce harmful effects on the viscera. Gradually, there occurs stagnation of foodstuffs which in time metamorphose into worms of various forms according to different combinations of the five elements" (Hoepli and Ch'iang, 1940). The Nei Ching besides giving the origins of helminths also described symptoms caused by the worms in general like "..... If one has a great number of short worms in the abdomen, one often dreams of crowds of people while one harbouring many long worms frequently dreams of fighting with other people and becoming wounded";

"Huang Ti asked: 'What causes salivation?' Ch'i Po replied: 'All things swallowed of course enter the stomach. There, heat is produced and it excites the worms to motion. The motion of the worm causes the stomach to relax and consequently the lien ch'uan (honest fountain) opens with the out-pouring of saliva' " and "..... Local heat in the part 3 or 4 inches below the rough portion of the elbow indicated presence of worms in the intestines" (Hoepli and Ch'iang, 1940). The Nei Ching emphasised the use of acupuncture as a form of anthelmintic treatment and it was performed in the following way: "..... short needles should not be used for the treatment of intestinal ascariasis. The symptoms of the disease are cardiac and intestinal pain, malaise, moving masses in the abdomen with intermittent pain, sense of heat in the abdomen with thirst and salivation. The moving mass should be caught and held fast by the palpating hands, and then punctured with a long needle. The needle should not be pulled out until those worms cease to move " (Hoepli and Ch'iang, 1940). Huang Ti thus represents the approach through sound methods of diagnosis. He is a symbol of the increasing reliance on pulse studies and on other physical signs,

as well as the development of physical therapy by means of massage, moxa, and exercise (Hume, 1940). The basic doctrines of Chinese traditional medicine were already evident 5000 years ago. The Yin-Yang Doctrine and the Five Elements Doctrine were part of the diagnosis and treatment. Traditional Chinese medicine, which existed since 1000 B.C. can be distinguished from primitive Chinese medicine by its self-consistent character separated from magical practices although the transition from early Chinese medicine to systematic Chinese traditional medicine was gradual.

Many of the philosophers who lived in the Chou Dynasty, which lasted from 1030 B.C. to 221 B.C. (Fitzgerald, 1969; Needham, 1975), deeply influenced the Chinese concepts of medicine. Chinese medicine developed greatly during this period (Wong and Wu, 1973).

Lao Tsu who was the keeper of the Imperial Archives of Loyang in the province of Honan in the sixth century B.C. (Feng and English, 1972), founded Taoism, Fung Yu Lan maintains that Taoism is "the only system of mysticism known which was not profoundly anti-scientific in its point of view" (Kao, 1973). Needham (1956) is of the same opinion. Taoism is incorporated into medicine since Ch'i ("energy") which signifies the original material substance, the "original nebula" or prime energy, motivated the Tao or Law of Nature (Palos, 1972). Fung Yu Lan (1966) related the similarity between the Taoist religion and the spirit of science by the common principle which is "the conquering force of nature".

Confucius (551 B.C. to 479 B.C.) was interested in the quality of human life. His philosophy, Confucianism, was the "main force in enabling the longest continuous feudal and semi-feudal society in world

history to resist basic structural changes" (Kao, 1973). It is probably also responsible for the conservative nature of Chinese traditional medicine which resulted from ancestral worship and filial piety.

Pien Ch'iao lived in approximately the fifth century B.C. (Palos, 1972). He diagnosed by inspection of the general physical state of the patient (Wang), by auscultation¹ and osphresiology² (Wen), by anamnesis³, including the patient's history (Wun) and by noting palpitation and sphygmology⁵ (Chieh). He was the first great physician in Chinese history. What he practised about two thousand years ago is still consistent with the fundamentals of Chinese traditional medicine in the twentieth century (Kao, 1973).

In ancient China, the two important lines of thought which attempted to interpret the structure and origin of the universe and all phases of human life were the Doctrine of Yin-Yang and the Five Elements Theory (water - fire - metal - wood - earth). The doctrine of the art of healing in ancient China postulated that there is a constant struggle in the human organism with its environment, as in Nature, between opposing and unifying forces. Good or bad health is determined by the fluctuations of these conflicting forces, the two polar forces being Yin and Yang. This philosophical doctrine was applied to malaria in the following extract: "Ch'i Po said: 'In malaria at the beginning of the attack, the Yang aura is amalgamated with the Yin. During this time, the Yang becomes weak while the Yin is vigorous and externally there is no protecting air so the patient feels chilly and trembles. When the Yin aura has functioned abnormally to the extreme, it will leave the Yang and come out. Then, Yang and Yin will again amalgamate in the outer part of the body. Consequently, the Yin becomes weak while

the Yang becomes strong so that the patient has now fever and feels thirsty.

If the aura of malaria is amalgamated with Yang, then the latter becomes stronger. If it is amalgamated with Yin, then Yin is stronger. While Yin is predominating, chill is caused. While Yang is predominating, fever is produced. Malaria is the aura of wind and cold which cannot last long so the patient will feel all right again when the disease has reached its climax' " (Hoepli, 1954a).

This Yin-Yang concept is again used in the treatment of helminthic infection by the massage method: "Fen (separate) yin yang one hundred times" (Hoepli and Ch'iang, 1940).

The Yin-Yang Doctrine is still being used to explain the theory of Chinese traditional medicine in modern China. The Five Elements Doctrine however, is in a less defined state. Medical authorities are not sure what should be done about this doctrine (Kao, 1973).

The association between man and his universe and the symbolic relation of parts of the body to the universe, like the analogy that head is heaven and the belly, the earth, are well-developed ideas in Chinese philosophy which greatly influenced the philosophy of Chinese medicine (Wallnofer and von Rottauscher, 1964).

The concept of Ch'i which literally means air or breath (vital energy) is important in the interpretation of the mechanism of acupuncture.

Huang Fu Mi (215 to 282) published the famous monograph on acupuncture Chia Yi Ching or the ABC of Acupuncture which was partially translated into English by Franz Hubotter, a German sinologist. In this classic treatise, he wrote: "Heartache caused by three kinds of

worms is attended by profuse salivation and inability to turn the body in the recumbent position. In this case, the pit of the stomach is the proper place for acupuncture' (Hoepli and Ch'iang, 1940).

When Wang Shu Mo (265 to 317) published the Classics of the Pulse, the development of acupuncture was aided greatly by this corresponding development of knowledge concerning pulse diagnosis. In Mo Chin Chen Pen or The Classics of the Pulse, it was stated that 'A pulse felt at the Chih (*R*) part to be under low tension and sliding in quality indicated that the patient has Tsun Pai Chung' (Hoepli and Ch'iang, 1940).

Around this period, several important herbs were discovered, one being ma-huang or Ephedra sinica.

While earlier documents like the Nei Ching advocated acupuncture and moxibustion⁶, 'Chun-yu' (circa 216), the Father of Clinical Medicine in China, used drugs. He used Daphne genkwa to expel worms and bitter ginseng as a mouthwash.

Chang Chi or Chang Chung Ching in the Han Dynasty (circa 217) wrote in his Synopsis of the Golden Chest Directly Explained, which was the most widely circulated medical book in China at that time: 'What is the distinguishing feature of the pulse of a case of abdominal pain caused by worms?' Somebody asked. The physician replied, 'During ordinary abdominal pain, the pulse becomes feeble and thready. If, on the contrary, it is full and bounding, it indicates the sure presence of Hwei Ch'ung (Ascaris) in the abdomen' (Hoepli and Ch'iang, 1940). The authorship of Shang Han Lun (Treatise on Fevers) was also attributed to him (Huard and Wong, 1968).

Hua T'o is recognized as the Father of Surgery in China. He

was born around 110 and died in around 207. He was skilled in treating diseases by using a few drugs and he knew precisely what quantities to prescribe. He used ta-suan (Allium scorodoprasum) with a mixture of vinegar as an anthelmintic for ascariasis (Huard and Wong, 1968).

Ko Hung (281 to 340) wrote a treatise on alchemy, dietetics, and magic called the Pao-p'u Tzu Nei-wai-p'ien. He advocated preventive medicine. He collated different old theories on the treatment of epidemic jaundice by mugwort, rhubarb, and gardenia, and malaria by Dichroa febrifuga as well as recording his own experiences of the use of sulphur and garlic for the prevention of tick-bites (Huard and Wong, 1968).

Ch'ao Yuan Fang (circa 610) is known for his work Ch'ao's General Treatise on the Etiology and Symptoms of Diseases. It is interesting to find in Ch'ao's above-mentioned work the following postulate: "The Pai Ch'ung reproduces by itself", indicating that Ch'ao Yuan Fang speculated self-fertilisation in tapeworms.

Li Shih Chen (about 1518 to 1593) compiled the Materia Medica. Needham stated that this was the only great scientific work written in the sixteenth century outside of the western scientific works of Copernicus, Vesalius, and Galileo. Porter Smith made the following observation on the Pen Ts'ao Kang Mu or the Great Herbal: "Many plants, having the characters 'Hu' (胡), 'I' (夷), or 'Hai' (海) are said to be of foreign origin. They were brought from Central Asia by such persons as the legate Chang Chien of the Han Dynasty, but are indigenous in a part of China proper. Many plants are acknowledged to be in some sense 'imports' " (cited in Wong and Wu, 1973). However, he did discover several new medicaments which are still used today. These

included Verbena officinalis, which was used against the malarial parasites; Leonurus sibiricus (motherwort), used in cases of dysmenorrhoea; Areca catechu, Chenopodium ambrosoides, Cyrtomium fortunei, Forsythia suspensa, Myrica lapidescens, Punica granatum and Quisqualis indica against helminths, and Rheum officinale, used for regulating blood pressure (Palos, 1972; Wallnofer and von Rottauscher, 1964). Ascaris had been used for therapeutic purposes as in the following example: "In treating chronic ophthalmia of long duration, in which the eyes have become red, use five Ascaris worms dried in the sun and ground into a powder. Add to it one mace of Ni fen (calomel powder) and half mace Shih tan (copper vitriol) in pulverized form and apply the mixture to the eye twice or thrice a day" (Hoeppli and Ch'iang, 1940).

Traditional Chinese medicine began to decline during the Ch'ing Dynasty when the Manchus ruled China. The Grand Medical College in 1882 gave an official order that acupuncture and moxapRACTICE be permanently suspended. In 1929, a resolution was passed to totally abolish traditional Chinese medicine. During this period, Chinese medicine might have been suppressed within China but the overseas Chinese continued to practise the traditional art of Chinese medicine.

Western medicine was formally introduced to China when the Christian missionaries arrived. The Rockefeller Foundation of New York contributed to the introduction of Western medicine to China (Wong and Wu, 1973). There were no drastic changes until 1949 when traditional Chinese medicine and Western medicine were "integrated" in China.

The period of the New Chinese Medicine is arbitrarily designated as beginning from 1949. Several events prior to this period,

however, ushered in this development.

In 1916, Liang Ch'i-ch'ao, the leading disciple of Yen Fu, helped organize a "Charitable Medical Society" whose purpose was to improve or reform medicine in China. This was to strengthen the race and through it the nation: "Preserving the people must start from medicine" (Croizier, 1976). Although from thence, China's progressive intellectuals viewed medicine as an important national concern, medicine had only a marginal effect on the promotion of economic or social modernization or nationalism before 1949. This was an era of frustrated hopes and unrealized plans (Croizier, 1974). This discrepancy between aspirations and reality made medicine a social, political, and even cultural issue in the intellectual life of that period. In 1944, the first political manifesto of traditional medicine was made. Caught in the transition between old and new, and East and West, China has had some characteristics of an ideological vacuum and others of an ideological melting-pot (Brandt, Schwartz, and Fairbank, 1967). In the midst of this social and cultural turmoil, Faust and Meleney published a monograph on schistosomiasis in 1924. Jang *et al.* (1946) investigated the efficacy of Ch'ang Shan, the roots of *D. febrifuga* as an anti-malarial treatment. More than one hundred million Chinese suffered from schistosomiasis, filariasis, malaria, leishmaniasis, hookworm infections, and possibly clonorchiasis, besides numerous other nutritional and infectious diseases. Hoeppli (1950-1) wrote a comprehensive article on the development of parasitology in China from 1930 - 1950.

When the Communists took power in 1949, one of their first priorities was the provision of more adequate medical care (Sidel, 1974). As a result, the pattern, rate, and some of the issues of medical

modernization changed radically. At a National Health Congress in Peking in the early 1950s, four principles were adopted, which remained the formulation on which medical policy is determined. They were:

- 1) Medicine must serve the workers, peasants, and soldiers.
- 2) Preventive medicine must be given priority over curative medicine.
- 3) Practitioners of Chinese traditional medicine must be united with practitioners of Western medicine.
- 4) Health must be integrated with mass movements (Sidel, 1974).

These policies were implemented as illustrated in the following account on schistosomiasis in China. In 1955, China asked the Japanese Society of Parasitology to advise them on the control of schistosomiasis. Professor Yochitaka Komiya spent six weeks in China and advised on using environmental control measures to eliminate Oncomelania. By 1958, 197 prevention and treatment stations with more than 12 000 medical teams were established in endemic areas and were managed by 17 000 specialized personnel. The "walking on 2 legs" policy, that is, making use of both Chinese and Western medicine, and adopting and adapting both traditional and modern practices, was implemented. The people were taught that schistosomiasis or the "big belly" disease is caused by a parasite rather than by "wind and water" (風水). Radio broadcasts, exhibitions, posters, lantern slides, dramatic performances, movies, and other mass media propaganda devices were used to disseminate information of parasitic diseases (Cheng, 1971). Health workers carrying microscopes went from door to door to show the people the different parasitic stages of Schistosoma japonicum. Besides displacing the soil to bury the snails, covering the river and lake banks with stones, constructing river banks with concrete material and

reclaiming land so as to eliminate Oncomelania by changing its environment, molluscicides like "tea-cake", which is the residue of the fruit of Thea (Camellia) oleosa, leaves of Schiva argentea and seeds from Croton tiglium, were used. Untreated night soil used to be a popular form of fertilizer. The treatment of human excreta was encouraged; the people were warned against using polluted water and polluting the water, and latrines were built.

The Great Proletarian Cultural Revolution occurred between 1967 and 1970. Of the many contradictions in this new society, the one that emerged most visibly then was the issue of high level scientific medicine on a narrow scale versus popular broad out-reaching medical programmes. Mao Tse Tung apparently supported the promotion of traditional medicine and its integration with modern medicine as evident from his general suspicion of elites and his emphasis on mass-based rural programmes (Croizier, 1976). With the onset of the Cultural Revolution, decentralization ensued. Many centres of training and research closed. Research in schistosomiasis stopped. Doctors were sent to the rural areas. The stress was on training local paramedical barefoot doctors, mass organization of rural medical cooperation and the drastic shortening of curricula in medical colleges. This resulted in a better distribution of health delivery care and probably stimulated more effective and more objective local control programmes. At the beginning of the Cultural Revolution, cases of schistosomiasis were increasing due to the disruption of the control programmed and research. However, a few years later, the mass of people were remobilized in the different local communities to eradicate the different parasitic diseases. It is difficult, if not impossible, to assess the accomplish-

ments and impact of the antischistosomal campaign in China but the effects of the fringe benefits of the campaign, like the betterment of environmental sanitation, repudiation of superstition, promotion of public education through the release of "scientific" information and the mobilization of the masses are obvious.

The "rediscovery of China" by Henry Kissinger in 1971 initiated a series of visits by foreigners to China and also kindled an interest in the Chinese culture. The academicians organized seminars and symposia on Modern China and Traditional Chinese Medicine (1972), Medicine in Chinese Cultures (1973), and the International Conference on Traditional Asian Medicine - its history, traditions, and integration into modern medical systems, was held in Australia in September, 1979. The Montreal Star (Saturday, January 7th, 1978) featured "A miracle was postponed" and "China: A Sleeping Giant" by Norman McCrae, adapted from the Economist. Robert Rodale, (1976) editor of Prevention "discovered" Tai Chi Ch'uan, the Chinese rhythmic dance exercise and the tau-fu, the soya bean-curd while "Looking For Chinese Health Secrets". World Health featured Traditional Medicine in its November 1977 issue. Dr. Bannerman of Ghana, who acted as the team leader to the group of community health specialists and administrators from 29 developing countries who met in Peking in August, 1977, suggested that the technique of acupuncture anaesthesia and herbal treatment developed by the Chinese be adopted by other countries. Kao (1973) also observed that the greatest advances in Chinese medicine are acupuncture anaesthesia and China's "new" pharmacology. Acupuncture is highlighted so greatly that most people equate acupuncture with Chinese medicine. Acupuncture is one aspect of traditional Chinese medicine, of which

herbal medicine forms the major component. Pergamon Press publishes The International Journal of Acupuncture and Electrotherapeutics. Most of the articles published in The American Journal of Chinese Medicine are on acupuncture. Although acupuncture is well-publicized, it is not generally accepted by most scientific circles. It is, however, gradually being recognized. Melzack (1973) wrote: "..... the phenomenon of acupuncture analgesia can be explained by the concept of a central biasing mechanism".

Time's Special Report on Socialism: Trials and Errors (March 13th. 1978) commented: "In China, the crash training of legions of doctors, nurses, and paramedics, and the funding of rural health centres have nearly eradicated cholera, plague, and other diseases that for centuries had periodically ravaged the population".

Not all reports, however, are as favourable as those mentioned above. Lasagna is optimistic about the "wedding" of traditional Chinese medicine and Western medicine, at the empirical level despite theoretical contradictions but the discrepancy between the staunch belief of the Chinese that Chinese traditional medicines are safe and the western pharmacologic principle that a material capable of doing good must also be capable of doing harm, made some Western scientists sceptical of traditional remedies (Lasagna, 1975). This can perhaps be resolved by the classification of Chinese medicaments. The Superior Class includes drugs like ginseng (Panax ginseng) and licorice root (Glycyrrhiza glabra) which are considered absolutely non-toxic and are used for a variety of diseases while the Common Drugs such as tang-kuei (Angelica sinensis) are slightly toxic, and their usage is limited only to certain diseases. The Inferior Drugs like ta-hwang (Rheum palmatum)

are very toxic and should be administered cautiously (Hou, 1977). Although traditional medicine is usually prescribed in China, the actual impact of it on the disease is difficult to assess.

Mary Brown Bullock (1977) reported in "The Key to Modernization" that the tension between the "reds" and "experts" had been eased. Although it is too early to identify the new trends in Chinese scientific and technological research, a "scientific renaissance" could be expected after Chairman Hua Kuo-feng's statement "May science and technology flourish and may the good news pour in". Some indications of the trends are reported below.

A follow-up report by Needham (1978a; 1978b; 1978c) noted the present atmosphere in China, summarized the phrase coined by Kuo Mo-Jo, "Springtime for the people, springtime for science". Vice Premier Fang Yi listed several policies which are necessary for encouraging the growth of science in China. The successful implementation of these policies - "There should be no bar on scientific publication apart from national security" and "The publication of scientific and technological results should be facilitated" - is already evident by the resumption of the publication of the following journals: Chinese Journal of (abbreviated as Chin.J.) Tuberculosis and Respiratory Diseases, Chin.J. Neurology and Psychiatry, Chin. J. Preventive Medicine, Chin. J. Stomatology, Chin. J. Obstetrics and Gynaecology, Chin. J. Radiology, Chin. J. Otorhinolaryngology, Chin. J. Physiological Sciences, Chin. J. Paediatrics, and Chin. J. Medical Laboratory Techniques. The international symposium on plants by anther culture, and the invitation of WHO personnel to China to discuss work on the pharmacology of new plant drugs are initial steps to concretize the policy

"The learning of advanced science and technology from other countries, and the increase of international academic exchanges" (Needham, 1978). Despite Needham's optimistic tones, one cannot help but notice their sense of priorities. The eight-year outline plan gives prominence to the fields of agriculture, industrial energy resources, materials, computer technology, laser optics, space science, high energy physics, and genetic engineering. Health and education are not given top priority. Several contradictions seem to exist (Carey, 1979; David, 1970). Chinese science and technology are furthest advanced in areas of their least need as reflected in this quote by a Chinese physician: "We tend to emphasise the sensational; reattaching several arms and fingers when what we need most is an effective public health programme". Although it had been observed by several visitors that infectious diseases are practically eliminated (Abelson, 1979), health statistics or any form of quantified evaluations to verify this are not available. The existence of several subtle contradictions in China seems to be the consensus of most of the visitors to China between 1977 and early 1979.

The "path" taken by the Chinese Medical Journal is an interesting one. From 1887 to 1966, the texts were in English. Its name was changed to China's Medicine in 1966 to 1968. Between 1969 and 1972, no volume of this journal was recorded in the Union List of Scientific Serials in Canadian Libraries (1978). The journal could be published but not sent overseas. This hiatus coincides with the period of the Cultural Revolution. It is most probable that the journals were prevented from being exported rather than that the publication ceased because the volume published in 1979 is numbered ninety-two. The name

Chinese Medical Journal was adopted again in 1973 but the texts were in Chinese with English abstracts. The new series was started in 1975 and the articles were in both Chinese and English. An analysis of the articles published proves to be illuminating. In 1975, the journal was saturated with political slogans and articles. Of the sixty articles in the volume published in 1976, 28 (47%) were on political ideology. In 1977, there was a drastic change; 56 (89%) of the sixty-three articles were medical articles. The first four issues of 1979 did not contain a single political slogan or political commentary. This illustrates the degree of control politics has over scientific research and publication in China.

Norman Bethune seemed to be an excellent reason for establishing relations between the Chinese Medical College of Peking and McGill University. The Norman Bethune Exchange Professorship was initiated by Dr. R.V. Christie, Dr. L. Stevenson, and Dr. Wilder Penfield in 1964. The first exchange professor was Dr. K.A.C. Elliott. The Norman Bethune Scholars of 1978 were Dr. Donald Bates and Dr. Raymond Prince. Dr. Bates (1978) noted that 30% of the curriculum of a medical student in China was devoted to traditional Chinese medicine. A Chinese delegation visited McGill University in 1978. The communication gap between China and the rest of the world seems to be closing.

Prior to 1949, medicine had only a marginal effect in the promotion of economic or social modernization or nationalism but a reassessment of the impact medicine has had on China today would probably indicate otherwise. The progress in the health care delivery in China, the advancement of traditional Chinese medicine and the integration of Chung-I (Chinese medicine) and Hsi-I (Western) medicine have

contributed greatly to the determination of the quality of life in China, which is dependent on social and economic conditions.

This section outlined the historical and philosophical background of traditional Chinese medicine. The hypotheses of the origin of parasites and their treatment fall within the constraints of this arena.

Footnotes:

1. Auscultation is a term in medicine applied to the method employed by physicians for determining, by the sense of hearing, the condition of certain internal organs. The ancient physicians appear to have practised a kind of auscultation, by which they were able to detect the presence of air or fluids in the cavities of the chest and abdomen (Thomson, W.A.R. 1974. Black's Medical Dictionary. 30th edition. pp. 73. London: Adam and Charles Black.)
2. Osmresiology is the scientific study of the sense of smell (The Compact Edition of the Oxford English Dictionary. 1977. pp. 2014. New York: Oxford University Press.)
3. Anamnesis is the term applied to the statement of the past history of some particular case of disease (Thomson, W.A.R. 1974. Black's Medical Dictionary. pp. 43. London: Adam and Charles Black.)
4. Palpitation is the condition in which the heart beats forcibly or irregularly and the person becomes conscious of its action (ibid. pp. 628.)
5. Sphygmology is the science of pulse-taking (sphygm(Greek) = pulse).
6. Moxibustion is cauterization by means of a moxa which is burning herb.

Section B : Hypotheses postulated on the origin of parasites.

Traditional Chinese medicine is culture-bound and an understanding of the philosophy of Chinese medicine and the hypotheses postulated on the origin of parasites are interrelated in developing the approach to this study. This may elucidate the rationale of Chinese anthelmintic treatment. By means of the Yin-Yang Doctrine, the Five Elements Doctrine, the belief in the symbolic relation of parts of the body in the universe, and the Ch'i Doctrine, the ancient Chinese arrived at two conclusions. Firstly, disease is rarely localized but usually affects the entire human being and secondly, disease is often associated with behaviour, manifested as a feeling of guilt caused by the infringement of a moral law (Veith, 1973). Assuming that the therapeutic value of Chinese herbal medicine lies in its chemical constituents, it should theoretically be possible to identify in each herb, the active ingredient or ingredients responsible for the "suspected" therapeutic effect. In practice, however, complications arise when an anthelmintic prescription which includes several herbs, is used. Synergistic and antagonistic effects are introduced. From the above concept of disease, the practical value of Chinese medicine that can be derived is the restoration or improvement of the general homeostatic mechanism or "health", rather than the action on a particular organ, system, or pathogen. It might thus be very difficult, if not impossible to correlate the therapeutic effect of an active compound with a specific change in a certain physiological parameter. Besides, the assumption that the chemotherapeutic effect of Chinese herbal medicine is the curative factor might not be valid. Other psychological and cultural

factors might be the effectors. In the treatment of parasitic infections, however, the rationale of treatment might be different if the traditional Chinese physicians, millennia ago, could equate the parasite as the causative agent of the disease or the manifestation of the disease.

The Doctrine of Spontaneous Generation and imaginary parasites, i.e. parasites described in Chinese medical texts which cannot be identified using the modern taxonomic classification system, played important roles prior to the advent of modern science. Spontaneous generation furnished the easiest and seemingly, the most obvious explanation accounting for the existence of intestinal parasites, especially the macroscopic larger worms which seemed to have grown from nothing (Hoepli and Ch'iang, 1950-1). Imaginary parasites had to be invented to account for the symptoms whose aetiologies were unknown (Hoepli, 1952).

Chinese classical medical literature frequently mentions nine kinds of worms of which only three can be identified with an acceptable degree of certainty. The existence of Ascaris lumbricoides (Huei Ch'ung, 蛔蟲), Enterobius vermicularis (Jao Ch'ung, 蟯蟲), and Taenia saginata or Taenia solium or Diphyllobothrium latum (Ts'un Pai Ch'ung, 寸白蟲) was known to the Chinese more than two thousand years ago (Hoepli, 1952). They believed that "Helminths are supposed to live either in the alimentary tract or in different organs, causing a great variety of disorders" (Hoepli and Ch'iang, 1938). The most popular hypothesis was that worms originated from a metamorphosis of various substances, especially of stagnating food. In certain ancient Chinese medical works, the writers were more specific, stating that

"heat" or "cold" produced by the stagnation of food interacted with "moisture" and that from this interaction, worms had their origin (Hoepli and Ch'iang, 1938). Multiple metamorphoses had also been postulated: Li Shih Chen wrote that "lice metamorphosed from the aura and afterwards they lay eggs, from which young lice are hatched," and "Jao Ch'ung (E. vermicularis) can metamorphose into many things of which the louse is one" (Hoepli and Ch'iang, 1953). It is also noted that several authors emphasised the necessity of avoiding raw and cold food, for example, meat and fish, and in dealing with Ts'un Pai Ch'ung (tapeworm), it was believed that the infection of this worm was caused by eating beef and drinking "white wine". A passage from Standards for Diagnosis and Treatment, written in 1602 by Wang K'en-t'ang reads "Man gets worms largely through irregular and careless eating; indulgence with raw minced fish, 'white wine', beef or mutton or the simultaneous consumption of turtle and spinach by mistake. The stomach becomes weakened so that the heat and moisture in the organ becomes irregularly regulated and as a result, worms such as Ts'un Pai Ch'ung develop" (Chu and Ch'iang, 1931; Hoepli and Ch'iang, 1938). This seems to indicate that the Chinese medical writers at least knew that T. saginata and D. latum could be transmitted by eating beef and raw fish, respectively. Some authors also pointed out the importance of weakened intestines with poor digestion in relation to the creation of helminths. Hoepli and Ch'iang found only one publication, The Three Causes and One Effect of Disease by Ch'en Yen in circa 1174, which postulated that worm infection is caused by the ingestion of progeny: "Some people become parasitized by worms through eating fruits and vegetables or animals' viscera, which contain their progenies" (Hoepli and Ch'iang, 1938).

It is a hypothesis well in advance of its time and scientific level. Several authors mentioned that the Ts'un Pai Ch'ung, the tapeworm, reproduced itself and therefore became rather long. It was also stated in Feng's Secret Records in the Embroidered Bag by Feng Chao Ching in 1702 that to effect a treatment successfully, the complete tapeworm had to be removed. The above suggestions were based on keen observation. The hypothesis which attributed "wind" 風 to be responsible for worm infection is based on philosophical speculation or mere imagination since the proponents of this hypothesis used the linguistic explanation, that the character "Feng" 風 (wind), composed of "fan" 凡 (all) and "chung" 蟲 (worm) were related, as supposed proof. In scabies, the mite was regarded not as the direct cause of scabies but its presence was a result of the disease. It was harmful and thus, its removal was desirable.

There are various interesting speculations on the behaviour of parasites which could affect anthelmintic treatment. The influence of the moon on intestinal parasites used to be common knowledge. Consequently, the phase of the moon was taken into consideration when prescribing anthelmintics (Hoepli, 1954). It was also believed that intestinal parasites had their heads turned upwards during the first half of the lunar month, and their heads' position reversed during the second half. Anthelmintic treatment thus, to obtain its full effect, had to be given during the first half of the lunar month; if not, the patient had to be given good food or be made to smell the odour of freshly roasted liver or meat, so as to entice the worm to face upward, prior to treatment (Hoepli, 1954).

These hypotheses indicate that the ancient Chinese thought

that helminths were the product of a weakened intestine, poor digestion, and improper eating. It is noteworthy that malaria according to classical Chinese medical texts, was caused by spirits and ghosts, climatic conditions and location, improper diet, the disharmony of Yin and Yang, and the irregular functioning of the Five Elements and the Mother of malaria, which could be termed recrudescence, or recurrence ("true relapse") in present terms. Hoeppli and Ch'iang (1938) stressed that many authors noted "the importance of a weakened intestine with poor digestion in relation to the creation of helminths, while there is a theoretical possibility that all people may have worms, it was believed that persons in good health with a good digestion are seldom parasitized". It would not be unreasonable to assume that most traditional Chinese anthelmintic treatments would emphasise "strengthening" of the intestine, improving digestion, or increasing peristaltic movement and "proper" eating. Some literature, however, did indicate that for the successful treatment of tapeworms, the complete cestode had to be removed. This would imply that the anthelmintic treatment would have to affect the parasite. Since helminths were believed, as mentioned earlier, to cause a great variety of disorders and might even cause death when they enter the heart or the kidney, the rationale would be to eliminate the parasite.

Section C : Traditional Chinese herbal anthelmintic prescriptions.

The hypotheses postulated by the Chinese to explain the origin of parasites did not change over a time period of two thousand years. A comparison of traditional Chinese herbal anthelmintics (abbreviated as TCHAs) used, indicates the same unchanging status quo. The twenty-four prescriptions listed below are selected from one hundred and twenty-one prescriptions used since 217 (A Barefoot Doctor's Manual, 1977; Chan, 1976; Chao, 1940; Hoeppli, 1959; Keys, 1976; Read, 1931). These reflect only the usual basic prescriptions used, since the Chinese physician tailors his treatment to fit the individual patient's condition as well as the nature of the disease (Hou, 1977). The prescriptions usually contain many medicinal substances which have various antagonistic, synergistic, and tonic effects (See Prescription 12). The observation that Chinese medicine emphasises complex mixtures of drugs is consistent with the holistic approach advocated by the principles of Chinese medicine.

The anthelmintic uses of the different herbs and the sources of citation of 121 traditional Chinese herbal anthelmintic prescriptions are tabulated (See Table 1) and their frequency of citation is enumerated (See Table 2). The subsequent selection of herbal materials used for experimentation was based on this and also on the availability of the herbs.

- A) These prescriptions were selected from Ch'ien Chin Fang (One Thousand Gold Prescriptions) by Sun Szu-Miao, about 650, during the T'ang Dynasty.

- 1) Mi Wu Wan - It was used for the treatment of ascariasis, which caused swelling in the abdomen of children. The symptoms were occasional abdominal pain, passing of white fluid, vomiting, fullness of the chest, chill and fever, pale and dry skin, malnutrition, and weakness of the limbs.

Mi-wu, <u>Selinum</u> sp.	藤蕪	8 fen ¹
Kuan-ching, <u>Aspidium falcatum</u>	貫眾	8 fen
Lei-wan, <u>Mylitta lapidescens</u>	雷丸	8 fen
Shen-chu-yu, <u>Evodia rutaecarpa</u>	山茱萸	8 fen
T'ien-men tung, <u>Asparagus lucidus</u>	天門冬	8 fen
Lang-ya, <u>Potentilla cryptotaenia</u>	狼牙	8 fen
Huan-lu, mushroom		4 fen
Kan-chu-hua, <u>Chrysanthemum sinensis</u>	甘菊花	4 fen

The above herbs were pulverized, mixed with honey, and rolled into pills. For 3 year-old children, 3 pills were given as a single dose. The dosage was increased accordingly up to a maximum of 10 pills.

- 2) This was a prescription for tapeworms.

Wu-yi, <u>Ulmus macrocarpa</u>	蕪荑	6 fen
Lang-ya, <u>P. cryptotaenia</u>	狼牙	4 fen
Pai-lien, <u>Vitis serjanifolia</u>	白欒	2 fen

The above materials were powdered and sifted. They were mixed with two kēh¹ of bitter wine overnight. This was to be taken on an empty stomach.

3) Another prescription for tapeworms is given below.

14 pieces of Ping-lang, Areca catechu (檳榔), were ground into powder and sifted. The shells of A. catechu were boiled in 2 1/2 sheng¹ of water until 1 1/2 sheng of the liquid was left. It was filtered and the powder was added. The solution was to be drunk from time to time. The patient had to lie in bed and be kept warm. The treatment was to be continued until all the worms were expelled.

B) Prescriptions 4, 5, and 6 were from Wai T'ai Mi Yao (The Private Prescriptions of an Official) which was written by Wang T'ao about 752.

4) This prescription was recommended for ascariasis.

Suan-shih-liu-ken, root of sour pomegranate, Punica granatum (酸石榴根), which extended eastwards 5 inches under the earth, cut into small pieces 2 sheng
A. catechu, pounded into small pieces 10 pieces

The above were decocted in 7 sheng of water. When 2 1/2 sheng remained, the residue was filtered out. A little rice was added to the filtrate and cooked into congee. This was to be taken in the early morning on an empty stomach. This remedy was very effective for killing worms and needed no precautions regarding diet.

5) This prescription was recommended for ascariasis which resulted in severe heartache and vomiting of 'clear

fluid".

The roots of Lien, Melia azedarach (楝), which had produced seeds were filed into fine powder. The powder was decocted with water and when the solution became brownish-black, it was filtered. The filtrate was used to cook congee. The patient was to fast the preceding night and early next morning. A piece of roasted meat was to be chewed and swallowed in order to induce the worms to turn their heads upwards. A little of the liquid was to be then taken gradually, increasing the amount from 1 spoonful to 1 1/2 sheng. The worms would be expelled at once.

6) This was recommended for cestodiasis.

Suan-shih-liu-ken, root of P. granatum, one handful of its branches directing eastwards.

Wu-i, U. macrocarpa

3 liang¹

Ch'ien-niu-tzu, powdered seeds of Ipomoea hederacea 牽牛子

1/2 liang

The first two herbs were decocted in 6 sheng of water. It was filtered and divided into 3 equal parts. The powder of the seeds of I. hederacea was added when the decoction was drunk. The successive doses were taken at intervals equal to the time required for a 5 li¹ walk. All the worms would be killed or passed out. The patient was to avoid eating raw or cold pork, beef, "white wine", K'wei (Malva verticillata, 葵) and suan (bamboo shoots, 笋).

- C) Ch'ung Chia Sheng Chi Tsung Lu (The Imperial Compilation of Medicine) compiled by the Imperial Officials at the order of the Emperor Hui-tsung of the Sung Dynasty about 1111 had this prescription.

7) This was suggested for the treatment of tapeworms.

Ping-lang, A. catechu 14 pieces

Chiu-ts'ai-pai, the white part of Allium odorum,
finely cut 薤菜白 1 cup¹

Yen-chih, salted beans 鹽豉 1 cup

A. catechu was pounded and sifted. The two other herbs were added and the mixture was divided into 3 equal parts. Each part was decocted with 3 cups of water until one cup remained. The residue was discarded and the solution was divided into 3 doses. No food was to be eaten during the preceding night. One dose was to be taken on an empty stomach in the early morning. The second dose was to be taken after an interval equal to the time required for walking 6 to 7 li. If the worms were not expelled, the final dose was to be taken.

- D) Some anthelmintic prescriptions from Yang Shih Chia Ts'ang Fang (Collected Prescriptions of Yang) by Yang Yen in the 5th year of Ch'un-hsi of the Sung Dynasty, about 1179, are listed here. Prescriptions 8, 9, and 10 were recommended for the treatment of worms (not specified) in children.

8) Lei Yuan San

M. lapidescens

Shih-chun-tze, Quisqualis indica, 使君子, fried and shells discarded

Ho-shih, Carpesium abrotanoides 鶴蝚

Fei-tze-jou, Torreya nucifera 榧子肉

A. catechu

Equal quantities of the above-mentioned herbs were pounded into fine powder. 1 ch'ien with diluted congee was taken as a single dose before feeding milk.

9) Hsung Che San

Hsung-huang, arsenic disulphide 雄黃 1/2 liang

She-~~si~~siang, musk 麝香 1 ch'ien

Kan-ch'i, juice of Rhus vernicifera, heated until no more smoke is produced 乾漆 3 ch'ien

Q. indica, fried and shells discarded 3 ch'ien

The above materials were pounded and mixed thoroughly.

One half ch'ien of the mixture made a single dose which was to be taken with a decoction of lien (M. azedarach) at any time.

10) Shih Chun Tze San

Q. indica, fried and shells discarded 20 pieces

U. macrocarpa, seeds grounded separately 1/2 liang

A. catechu 1 piece

Ta-fu tze, Areca dicksonii 大腹子 2 pieces

The herbs were pounded into fine powder. The seeds of U. macrocarpa were added and ground together. 1 to 2 ch'ien made a single dose which was to be taken with cooked pork soup before milk-feeding.

- E) Hsu Ch'un-fu, in 1556 (the 35th year of Chan-ching of the Ming Dynasty) compiled Ku Chin Yi T'ung Ta Ch'uan (General Medicine of the Past and Present). One of the prescriptions from it is listed below.

- 11) Hua Tu Wan was a dissolving poison pill used for treating worm diseases of adults and children.

Seeds of <u>C. abrotanoides</u>	1 liang
<u>A. catechu</u>	1 liang
Root of <u>M. azedarach</u> , cortex discarded	1 liang
Lead carbonate, 胡粉 fried	1 liang
Alum, 白礬 burned	2 1/2 ch'ien

The ingredients were ground into fine powder which was to be mixed with water and rolled into pills as big as Ma-tze (麻子). 15 pills were recommended for an adult and 5 pills for children. This was to be taken on an empty stomach.

- F) Prescriptions 12 and 13 were prescriptions given by "old-style practitioners" in Peking in 1940 (Chao, 1940).

- 12) This was a general prescription for the treatment of worm diseases.

Chiao-shen-ch'u, roasted spirit leaven, 焦神曲 2 ch'ien

Ch'ao-mai-ya, baked bud of Hordeum vulgaris,

炒麥牙 2 ch'ien

Ch'ing-p'i, unripe peel of Citrus nobilis, 青皮 1 ch'ien

Ch'en-p'i, peel of C. nobilis, 陳皮 1 ch'ien

San-leng, Scirpus maritimus, 8 fen

O-chu, Kaempferia pandurata, 莪朮 8 fen

Ch'uan-lien, Coptis teeta, 川連 5 fen

A. catechu 5 fen

Lu-huei, Aloe vulgaris, 蘆薈 5 fen

U. macrocarpa 1 1/2 ch'ien

Q. indica 1 1/2 ch'ien

Dr. Chao, who prescribed this, explained that S. maritimus and K. pandurata stimulated the activity of the blood and dissolved the clotted blood; U. macrocarpa, Q. indica, ripe and unripe peels of C. nobilis increased the appetite and regulated the breath of the patient; the roasted spirit leaven and H. vulgaris also increased the appetite and helped digestion; A. vulgaris and C. teeta regulated the functioning of the liver and helped ease the fever. This remedy illustrates the integrated nature of Chinese therapeutics. The drugs were taken twice daily in the form of a warm decoction. The patient was advised against taking raw, cold, and indigestible food. If the above prescription was not effective, the following prescription was to be taken.

13) Hua Ch'ung Wan

<u>C. abrotanoides</u>		1 liang
Yuan-ming-fen, sodium sulphate,	元明粉	1 liang
<u>M. azedarach</u>		1 liang
<u>A. catechu</u>		1 liang
<u>U. macrocarpa</u>		1/2 liang
Alum,	枯礬	1/2 liang
<u>Q. indica</u>		1/2 liang

The ingredients were pounded into fine powder and rolled with honey into pills. This pill was recommended for the treatment of the following diseases: worm disease of either children or adults with symptoms of yellow face, heart, and stomach pains, poor appetite, discomfort in sleep, and the presence of white plaques on the face.

G) The geographical extent within which traditional Chinese medicine is still being practised today by Chinese communities is illustrated by Prescriptions 14. to 24.

14) This anthelmintic prescription was prescribed by a Chinese traditional physician from Penang, Malaysia in 1975.

<u>Anomum xanthiodes</u> ,	砂仁	5 ch'ien
<u>A. catechu</u>		2 ch'ien
<u>Q. indica</u>		3 ch'ien
<u>U. macrocarpa</u>		3 ch'ien

<u>C. nobilis</u>		5 ch'ien
<u>T. nucifera</u>		3 ch'ien
<u>C. abrotanoides</u>		3 ch'ien
fowl's gizzard, 鷄腎		3 ch'ien

The above was to be boiled in 1 1/4 cups of water until eight-tenth (= 1 cup) of it remained. The decoction was to be drunk only after fasting for a few hours.

- 15) Chinese Herbs: Their Botany, Chemistry, and Pharmacodynamics by Keys was published in 1976. Most of his prescriptions were collected from Chinese medical dispensaries in San Francisco. Prescriptions 15 to 17 are selected from this book. Prescription 15 is recommended for ascariasis.

P. granatum
M. azedarach
A. falcatum
A. catechu

Equal amounts of the herbs were combined and powdered.

A dose of 7.5 - 10.0 g was to be taken before breakfast.

- 16) This was recommended for enterobiasis.

Torreya grandis, 榧子
A. catechu
U. macrocarpa

Equal amounts of the above-mentioned were combined and powdered. A dose of 10 g was to be taken before breakfast.

17) This was prescribed for hookworm infections.

<u>Q. indica</u>	10.0 g
<u>P. granatum</u>	2.5 g
<u>A. catechu</u>	2.5 g

The herbs were combined and powdered. A dose of 7.5 g was to be taken before breakfast.

18) Prescriptions 18 to 24 were selected from A Barefoot Doctor's Manual (1977). This prescription was recommended for ascariasis.

M. azedarach, root bark well-scraped of its outer covering
5 ch'ien to 1 liang

A concoction of the herb was made by decocting it for 3 to 4 hours down to 60 to 80 ml. This was to be taken on an empty stomach every morning, 2 mornings in a row.

19) This is another prescription for ascariasis.

Q. indica, roasted and shelled 1 liang

3 ch'ien was to be taken on an empty stomach, each time, chewing well before swallowing. This was to be repeated 3 mornings in a row. A 3-day break was to follow. The treatment was to be repeated.

20) This is recommended for cestodiasis.

A. catechu 1 to 4 liang

A concoction of the herb was to be taken cold.

- 21) This is also prescribed for cestodiasis.

Pumpkin seeds, powdered

4 liang

A dose of the above-mentioned amount was to be taken at one time. It was to be followed by Glauber's salt, 3 hours later.

- 22) 3 ch'ien powdered Lui-yin, Omphalia lapidescens (probably also known as M. lapidescens - author) was also recommended for cestodiasis.

- 23) This is a preparation for enterobiasis.

A dose of 3 fen powdered Q. indica was recommended for each year of a child. The maximum dose is 1 ch'ien and 2 fen. This was to be taken three times a day with a decoction of 3 ch'ien Pai-pu (Stemona sessifolia). This was to be repeated 6 days consecutively.

- 24) This is another prescription for enterobiasis.

A. catechu

3 ch'ien

T. nucifera

3 ch'ien

Black prune, Prunus mume, 烏梅

3 ch'ien

A concoction of the above was to be taken by the patient.

Most of the 121 traditional Chinese anthelmintic prescriptions studied were selected from Chao (1940). He translated several anthelmintic prescriptions from the Han Dynasty (about 217) to 1940. He identified each prescription with the period in which it was written.

This is indicated in the Source of Citation (See Table 1) so as to give an overview of the time span in which the prescriptions were written.

The anthelmintic prescriptions studied could be a biased selection by the different authors but the sample of 181 prescriptions screened from various sources should be a fair representation of Chinese anthelmintics. Table 2 indicated that A. catechu, M. lapidescens, U. macrocarpa, Q. indica, M. azedarach, P. granatum, and C. abrotanoides are the most frequently mentioned, in that order.

The prescriptions indicated that some herbs are frequently prescribed for specific parasites. M. azedarach is more often prescribed for ascariasis while A. catechu seemed to be more popularly used against cestodes.

The herbal anthelmintics selected for the laboratory evaluation were A. catechu, M. lapidescens, U. macrocarpa, Q. indica, and M. azedarach. Since the anthelmintics were selected, a review of the current literature in English on the laboratory evaluation of the efficacy of traditional Chinese herbal medicine would prove useful.

Footnotes:

1. See Appendix 1: Conversion Tables.

D

Table 1: The anthelmintic use of herbs and their
sources of citation (121 Chinese prescriptions
surveyed)¹.

O

HERB	ANTHELMINTIC USE OF HERB	SOURCE OF CITATION
A) <u>Areca catechu</u>	1) Broad-spectrum anthelmintic (18)² 2) Against <u>Ascaris lumbricoides</u> (4) 3) Against <u>Enterobius vermicularis</u> (2) 4) Against hookworms (1) 5) Against¹ cestodes (9)	1) a) Chao (1940) - The prescriptions were dated from 752 to 1940. 2) a) Chao (1940) - The prescriptions were dated from 752 to 1179. b) Keys (1976) 3) a) Chao (1940) - The prescription was written in 1111. b) Keys (1976) 4) a) Keys (1976) 5) a) Read (1931) b) Chao (1940) - The prescriptions were dated from 650 to 1556. c) Keys (1976) d) A Barefoot Doctor's Manual (abbreviated as BDM) (1977) (next)

1. See text for explanation.

2. The number (in parentheses) of prescriptions which included the herb used for the particular usage cited.

Table 1 (cont.)

HERB	ANTHELMINTHIC USE OF HERB	SOURCE OF CITATION
B) <u>Melia azedarach</u>	1) Broad-spectrum anthelmintic (9) 2) Against <u>A. lumbricoides</u> (5) 3) Against <u>E. vermicularis</u> (1) 4) Against cestodes (1)	1) a) Chao (1940) - The prescriptions were dated from 650 to 1940. 2) a) Chao (1940) - The prescriptions were dated from 650 to 1111. b) Keys (1976) c) BDM (1977) 3) a) Chao (1940) - The prescription was written in 1111. 4) a) Chao (1940) - The prescription was written in 752.
C) <u>Mytilus lapidescens</u>	1) Broad-spectrum anthelmintic (15) 2) Against <u>A. lumbricoides</u> (1) 3) Against <u>E. vermicularis</u> (1) 4) Against cestodes (5)	1) a) Chao (1940) - The prescriptions were dated from 650 to 1940. 2) a) Chao (1940) - The prescription was written in 1179. 3) a) Chao (1940) - The prescription was written in 752. 4) a) Read (1931) b) Chao (1940) - The prescriptions were dated from 650 to 1179. c) Keys (1976) d) BDM (1977) (next)

Table 1 (cont.)

HERB	ANTHELMINTHIC USE OF HERB	SOURCE OF CITATION
D) <u>Quisqualis indica</u>	1) Broad-spectrum anthelmintic (11) 2) Against <u>A. lumbricoides</u> (2) 3) Against <u>E. vermicularis</u> (1) 4) Against hookworms (1) 5) Against cestodes (2)	1) a) Read (1931) b) Chao (1940) - The prescriptions were dated from 1260 to 1940. c) Chan (1975) 2) a) Chao (1940) - The prescription was dated 1179. b) BDM (1977) 3) a) BDM (1977) 4) a) Keys (1976) 5) a) Chao (1940) - The prescriptions were dated 1260 and 1179. (next)

Table 1 (cont.)

HERB	ANTHELMINTHIC USE OF HERB	SOURCE OF CITATION
E) <u>Ulmus macrocarpa</u>	1) Broad-spectrum anthelmintic (14) 2) Against <u>A. lumbricoides</u> (1) 3) Against <u>E. vermicularis</u> (3) 4) Against cestodes (3)	1) a) Read (1931) b) Chao (1940) - The prescriptions were dated from 650 to 1940. c) Chan (1975) 2) a) Chao (1940) - The prescription was written in 1111. 3) a) Chao (1940) - The prescription was written in 650. b) Keys (1976) 4) a) Chao (1940) - The prescriptions were dated from 650 to 752.
F) <u>Punica granatum</u>	1) Broad-spectrum anthelmintic (5) 2) Against <u>A. lumbricoides</u> (2) 3) Against hookworms (1) 4) Against cestodes (5)	1) a) Chao (1940) - The prescriptions were dated from 1111 to 1260. 2) a) Chao (1940) - The prescription was written in 752. b) Keys (1976) 3) a) Keys (1976) 4) a) Read (1931) b) Chao (1940) - The prescriptions were dated from 752 to 1556.

(next)

Table 1 (cont.)

HERB	ANTHELMINTHIC USE OF HERB	SOURCE OF CITATION
G) <u>Carpesium abrotanoides</u>	1) Broad-spectrum anthelmintic (10) 2) Against <u>A. lumbricoides</u> (1)	1) a) Chao (1940) - The prescriptions were dated from 1260 to 1940. b) Chan (1975) 2) a) Chao (1940) - The prescription was written in 752.
H) <u>Torreya nucifera</u>	1) Broad-spectrum anthelmintic (3) 2) Against <u>E. vermicularis</u> (1) 3) Against cestodes (2)	1) a) Chao (1940) - The prescription was written in 752. b) Chan (1975) 2) a) BDM (1977) 3) a) Read (1931) b) Chao (1940) - The prescription was written in 752.
I) <u>Evodia rutaecarpa</u>	1) Broad-spectrum anthelmintic (4) 2) Against <u>A. lumbricoides</u> (1) 3) Against <u>E. vermicularis</u> (1) 4) Against cestodes (4)	1) a) Read (1931) b) Chao (1940) - The prescriptions were written between 752 and 1111. 2) a) Read (1931) 3) a) Chao (1940) - The prescription was written in 1111. 4) a) Read (1931) b) Chao (1940) - The prescriptions dated from 752 to 1111.

Table 2: The number of citations of herbs (121 Chinese prescriptions surveyed).

HERBS	BROAD-SPECTRUM ANTHELMINTIC	MENTIONED SPECIFICALLY FOR NEMATODES				MENTIONED SPECIFICALLY FOR CESTODES	TOTAL NUMBER OF PRESCRIPTIONS WHERE HERB IS CITED
		<u>A. lumbricoides</u>	<u>E. vermicularis</u>	Hookworm	TOTAL FOR NEMATODES		
1) <u>A. catechu</u>	18	4	3	1	8	9	35
2) <u>M. lapidescens</u>	15	1	1	0	2	5	22
3) <u>U. macrocarpa</u>	14	1	3	0	4	3	21
4) <u>Q. indica</u>	11	2	1	1	4	2	17
5) <u>M. azedarach</u>	9	5	1	0	6	1	16
6) <u>P. granatum</u>	5	2	0	1	3	5	13
7) <u>C. abrotanoides</u>	10	1	0	0	1	0	11
8) <u>E. rutaecarpa</u>	4	1	1	0	2	4	10
9) <u>T. mucifera</u>	3	0	1	0	1	2	6

Section D : Current work on the efficacy of traditional Chinese herbal medicine.

— A search for scientific literature available on experiments dealing with animal models treated with Myrica lapidescens, Melia azedarach, and Ulmus macrocarpa, led to a cul-de-sac. Most of the published works on Chinese medicine is concerned with acupuncture and herbs like Panax ginseng, ginseng (Rim, Sang, Pyung, and Jang, 1970), Gardenia jasminoides, gardenia (Koo and Li, 1977), Anisodus tanuticus (Li, 1974; Li, 1975), and Leonurus sibiricus, motherwort (New Evening Post, 1978; Yun, Hu, Lau, Che, Yeung, Cheng, and Hwang, 1976). There is hardly any experimental work done on Chinese anthelmintics other than Areca catechu, areca nut (Chandler and Chopra, 1928, cited in Hoeppli, 1959; Chang, 1950-1; Chung, Hou, Huang, and Wang, 1951; Feng, 1950; Feng, Liu, Ting, Hwang, 1949; Hall and Foster, 1918; Hall and Shillinger, 1923; Liu, 1936), Cucurbita pepo, pumpkin seed (Chou and Ming, 1960); the two afore-mentioned combined (Chung and Ko, 1976), P. ginseng (Rim et al., 1970) and Quisqualis indica (Chen and Hou, 1926). It is obvious that recent scientific studies on the laboratory evaluation are rare. The limited published work on Chinese traditional anthelmintics is largely clinical case reports. The active parasitoses being researched in China now are schistosomiasis, filariasis, and clonorchiasis. Active research work in traditional Chinese medicine regarding schistosomiasis and other endemic diseases was to have begun in China, for example, at the Institute of Materia Medica in Peking and the Shanghai Institute of Materia Medica (Hou, 1977) by 1959 (Chinese Medical Journal, 78: 103-105, 1951; 78: 205-209, 1959;

80: 208-213, 1960) but it was recently reported that "Chinese scientists are just beginning to examine some 1000 herbal remedies" (Report of the American Schistosomiasis Delegation To The People's Republic of China, 1977).

Most of the information is not exported out of the People's Republic of China. Chung and Ko (1976) treated 33 patients infected with Taenia saginata with areca nuts and pumpkin seeds. No mention of the cure rate was made in the paper. In all of the clinical testing of Chinese anthelmintics encountered, the placebo and double-blind trials were not used. The studies were carried out in Chinese communities. This point is emphasised since psychological and cultural factors are very important in this situation.

In The Puzzle of Pain by Melzack (1973), it was noted that in experiments on "audio analgesia", intense auditory stimulation accompanied by strong suggestion that it eliminates pain produced a significant increase in pain-tolerance duration compared with the control condition without auditory stimulation. Intense auditory stimulation without explicit suggestion or strong suggestion accompanied by the elaborate placebo stimulus but without intense auditory stimulation did not increase pain-tolerance durations compared with the control condition. This seems to indicate that even in Western societies today, the psychological factor, as in the above-mentioned case, in hypnosis and psychosomatic diseases, and cultural factors are important in setting not only the threshold but also the context of pain (Blakemore, 1977) without the added complication of an interaction of cultural, social, philosophical, and political experiences that seem to play a major role in Chinese medicine. Although one could argue that psychological disorders,

such as pain are not directly comparable with parasitic infections, one should be aware of the practical application of Chinese thought to Chinese medicine, that is, it is primarily interested in function as opposed to substratum (Porkert, 1976).

The traditional Chinese doctors are not concerned with, for example, the reduced incorporation of protein precursor into the muscle as an effect of gastro-intestinal parasitic infection (Soulsby, 1976) but rather in the obvious qualitative changes (Porkert, 1974) which often result in pain and thus are ultimately, dependent on the mind. Although caution is needed in extrapolating results from studies done on animal models to human beings, these studies are carried out since traditional Chinese anthelmintic treatment could slowly become folk medicine if left uninvestigated. Experiments on animals, where the psychological factor is eliminated, are now being actively pursued in China and Japan.

In 1962, James Chen Y.P. reviewed the available literature on pharmacology in mainland China and the materials surveyed were from the Chinese Medical Journal, the Chinese language periodical Yao-hsueh T'ung-pao (Pharmacology Bulletin), Acta Pharmaceutica Sinica and Acta Physiologica Sinica. In the 1950s, the primary emphasis in chemotherapy and traditional Chinese medicine was directed towards eradicating parasitic and infectious diseases. Although Chen (1962) wrote: "With the exception of some of the studies on parasitocidal agents, the great majority of trials with traditional medicine were conducted on a relatively small number of patients and more important, without proper controls, reference standards, adequate follow-up studies and quantitative evaluation of drug action". The articles by Hsien Hsing Min (1959) and

Chung and Ko (1976), which were studies on parasiticidal agents, were clinical reports and other experiments performed on humans were not done with placebo or double-blind trials. In the field of parasitology, the need for more rigid scientific studies on traditional Chinese medicine is wanting, although Chen (1962) seemed more optimistic. The most common traditional Chinese anticestodal treatment investigated is the combined use of C. pepo and A. catechu. It was reported that the dried seeds of pumpkin Cucurbita moschata, have been demonstrated to have "striking therapeutic effects" in the treatment of acute schistosomiasis (Cheng, 1971). Rhizomes and roots of Rubia cordifolia with A. catechu and Hemerocallis thunbergii are used in the early stages of the disease while a mixture of the following plant materials, Pachyma cocos; rhizomes of Atractylis ovata and Menispermum dauricum; barks of Magnolia officinalis, Eucommia ulmoides, and Cinnamomum loureirii; roots of Angelica polymorpha; tubers of Alisma plantago and seeds of Citrus nobilis, is used to help restore splenic functions, facilitate passage of nitrogenous wastes and remove excess body fluids (Cheng, 1971). It is surprising that no work had been done on the investigation of the efficacy of M. azedarach, Carpesium abrotanoides, M. lapidescens, Punica granatum, and U. macrocarpa as anthelmintics. These are the most frequently-mentioned anthelmintics in traditional Chinese medical literature and recent books on traditional Chinese medicine.

In 1973, Li C.R. spent two months in the People's Republic of China. He learned that Chinese biomedical scientists have made remarkable advances in the field of traditional herbal medicine during the past 20 - 25 years, utilising traditional Chinese medicine as a rich source of potential agents. His monograph aims at encouraging Western

biomedical scientists to consider "new" ideas from China although he does not advocate going back to folk medicine nor encouraging people to use "great-grandmother's remedies". He emphasises that thorough investigation must be carried out before herbs can be used safely and effectively by the health professionals. In the Report of the American Schistosomiasis Delegation To The People's Republic of China (1977), it was noted that Chinese scientists are beginning to examine herbal remedies (many are mixtures) in the following way: They are first extracted with water, alcohol, or other solvents and then administered to mice as a crude concentrate. Li (1974) observed that when a physician has written a prescription, it is brought to a "drug store" where the herbs are measured, according to the Chinese system of measurement and put into a package. The herbal material (often animal material is also included) is boiled in a non-metallic utensil for a specified length of time in a prescribed amount of water. The purpose of boiling herbs in water is to provide a specific amount of extract which the patient is required to drink.

Li (1974) also reported on some recent experimental studies and clinical applications of traditional Chinese medicine. A pharmacognosy of individual herbs discussed in his monograph was also included. Most of the workers are aware that the problems of studying the pharmacology of traditional Chinese medicine are numerous. The climatic conditions, soil, and terrain where the plants are grown, all affect their chemical constituents and thus their medicinal properties. Various parts of the medicinal plants are used for different therapeutic purposes. The roots could have anthelmintic properties while the seeds could affect hypotensive action. Despite the immense number of diffi-

culties associated with the investigation of Chinese herbal medicine, it should not be ignored because of its potential value, and contemporary significance to almost a third of the world's population.

Awareness of the utilisation of traditional medicine, which has evolved adaptively within a cultural setting, is increasing. Consequently, ethnomedicine and medicinal plant research are encouraged. To allay and overcome the fears of the limitations of modern medicine, traditional medicine could complement (Forkert, 1970) rather than substitute for, modern medicine, promoting the concept of World or oecumenical medicine (Kao, 1979).

The present study of traditional Chinese herbal anthelmintics is a modest endeavour directed at this goal.

Section E : Discussion of Part I.

Tracing the development of traditional Chinese medicine reveals the complex relationship between natural philosophy and medicine, and the evolution of the "Chinese rationality". It showed that Chinese medicine is not based on magico-religious concepts but rather on empiricism. A review of the hypotheses formulated by the Chinese to explain the origin of helminthic infections indicated keen observation. The Chinese could relate the helminthic infections to the food they ate even though the causal agent was not known. The universal Doctrine of Spontaneous Generation with different variations was also adopted by the Chinese. Early Chinese authors had also observed that pinworms migrated out of the anus at night and that lice left the body after the host died (Hoeppli, 1952). They were more interested in symptoms rather than the causal factors, as in Western medicine. Consequently, although the causative agents were not known, the clinical picture of severe infections with hookworms, schistosomiasis, and malaria were well-documented. The traditional Chinese herbal anthelmintics screened were consistent with the holistic approach advocated by traditional Chinese medicine. They were also consistently similar over a period of two thousand years. The current work on Chinese medicine in China seemed to be affected by politics, and socio-economic and cultural factors.

This section forms the theoretical framework of the dissertation. Traditional Chinese medicine evaluated on its theoretical expositions and historical documentation would appear to be sufficiently efficacious to warrant a laboratory evaluation. The hypotheses of the origin of parasites are disproved but the efficacy of the treatments,

like so many other aspects of traditional Chinese medicine, have yet to be tested using the western Scientific Method.

An analysis of the theoretical orientations in Chinese medical parasitology reveals that there are sufficient grounds to base a laboratory evaluation of traditional Chinese herbal anthelmintics.

The components of the total effect of traditional Chinese herbal anthelmintics (E_T) include the following, which become increasingly restrictive :

- E_n = the environmental component
 - = $A + B$ (where A = abiotic or physical factors
 B = biotic factors),
- S = the human social component,
- C = the cultural component,
- P_s = the psychological component,
- P_h = the physiological component, and
- T = the chemotherapeutic component.

The components may interact with each other in various ways, e.g. synergistically, antagonistically, additively, subtractively, or independently.

The evaluation of Part I measures E_T . Part III evaluates only T , quantitatively.

PART II

Section A : The Scientific Method

The distinguishing feature of science is its method. The problem of defining the Scientific Method, however, is far from resolved. Some argue that it is not a method but, rather several methods.

A brief working definition of the Scientific Method : "the application of logic and objectivity to the understanding of phenomena" (Kempthorne, 1952) will suffice as the starting point of this discussion. The Scientific Method can be broadly outlined as 1) the formulation of hypotheses, and 2) the testing of the hypotheses. These processes are illustrated by the experience of Bateson (1976) : "the advances in scientific thought come from a combination of loose and strict thinking".

Some of the controversy over the Scientific Method arises from the disagreement over the methods of the application of logic and objectivity. Popper (1968a; 1968b) advocated that the scientific procedure should be hypothetico-deductive rather than inductive. The Popperian approach proposed falsifiability as a criterion of demarcation distinguishing between the empirical sciences on the one hand, and mathematics and logic, as well as the "metaphysical" systems, on the other (Popper, 1968a). Popper's concept of the growth of scientific knowledge thus, was not the accumulation of observations but the repeated refutation of scientific theories and their replacement by better or more satisfactory ones (Popper, 1968a) which coincides with Kuhn's concept of paradigms and the structure of scientific revolutions (Kuhn, 1970). Weatherall (1969) also agreed that inductive inference is logically unsound. Pelto and Pelto (1978) applied the Popperian principle to anthropological studies, advocating that hypotheses must be falsifiable.

Waddington (1977) however, disagreed with Popper on the point that the effective use of the Scientific Method depended on the falsifiability of hypotheses. His requirements, which included the ability to ask important questions and the ability to devise experiments which gave unambiguous answers, were less stringent than that of Popper's.

Another point of controversy is the term "the application of objectivity" attributed to the Scientific Method. The ideal of scientific detachment is rejected by Polanyi (1964) who postulated the concept of Personal Knowledge. The ambiguity of the term "objectivity" is also illustrated by the "searchlight theory of science" which states that a scientific description will depend greatly on the point of view and interests of the experimenter, although it will also depend on the facts described (Davis, 1973; Popper, 1971). Although many of the opinions expressed are personal, the importance of the Scientific Method is basically its awareness of its limitations (Popper, 1974). It is noted that while the Scientific Method is widely applied in Western research, and while its value is not seriously challenged, several philosophers differ in their emphases and in their evaluation of its various approaches.

Bertalanffy (1968) attempted to rectify the reductionistic approach of the Scientific Method by suggesting a more holistic approach called the "General Systems Theory".

The design of experiments is an aspect of the Scientific Method. An experiment could be arbitrarily divided into 3 sections: 1) The Experiment, 2) The Design, and 3) The Analysis. The Experiment involves the statement of the problem and the formulation of the hypotheses. The dependent variables are selected. In the design, the

number of observations to be taken, order of experimentation, and the method of randomisation to be used are determined. The three considerations that have to be taken into account in the design of experiments, i.e. the experimental conclusions must have validity, precision, and wide coverage, all emphasise randomisation, replication, and heterogeneity. The "controlled experiment" is considered as one of the most important concepts in biological experimentation (Beveridge, 1968).

The biological assay, which is a method for estimating the potency (effect) of a material by means of the reaction elicited in living organisms, is one of the important tools in chemotherapeutic research. The economical and other practical limitations of experimental designs have to be considered too. The analysis consists of data collection and processing, computation of test statistics, and the interpretation of the results. Since biological variation exists, statistical analysis is essential. It will not provide any absolute proof to the experimenter of the effectiveness of the treatments but will enable the experimenter to estimate the reliability of the results. Several books and articles expound on experimental designs and statistical analysis, related to biological problems (Cochran and Cox, 1950; Duncan, 1955; Federer, 1955; Finney, 1955; Finney, 1971; Fisher, 1971; Hicks, 1964; John and Quenouille, 1977; Kempthorne, 1952; Steel and Torrie, 1960). The articles by Bliss (1968), Pauls (1968), and Waitz (1968), specifically dealt with statistical analysis of data in chemotherapeutic research.

In the laboratory evaluation of traditional Chinese herbal medicine, I have attempted to apply some of the principles of the Scientific Method, Experimental Design, and Statistical Analysis, discussed above. The application will be discussed in the Discussion (Part III).

Section B : The application of some pharmacological principles to the chemotherapy of gastro-intestinal helminthiases.

Pharmacology may be defined as the study of the selective biological activity of chemical substances on living matter (Bevan, 1976). A substance has biological activity when in small doses it initiates cellular and subcellular changes. It is selective when the response occurs only in some cells.

Most of the chemicals used in chemotherapy have selective biological activity, hence, the relevance of pharmacology in chemotherapeutic research. This section will emphasise the chemotherapy of gastro-intestinal helminthiases since the parasite models used in the laboratory evaluation were gastro-intestinal parasites.

Anthelmintics act by affecting specific sites or systems of the parasite, host, or both. The drug-receptor hypothesis accounts for the phenomenon of specificity. The mechanism of the mode of action, however, varies with different drugs. It can have a biochemical effect, as seen in the inhibitory effect of trivalent organic antimonials (eg. stibophen) on schistosomal phosphofructokinase (Van den Bossche, 1976). Piperazine was found to reduce succinate formation, which consequently resulted in the paralysis of muscles of Ascaris lumbricoides (Saz and Bueding, 1966; Van den Bossche, 1976).

Some aspects to be considered in the chemotherapy of helminthiases :-

- 1) A parasitic infection is not necessarily equated with clinical disease. The number of parasites, the resistance and nutritional status of the host, and other biological factors, interact to determine

the manifestation of clinical disease.

- 2) The treatment of parasitic diseases is often complicated by drug toxicity, mixed infections, Jarisch-Herxheimer reactions to a massive release of helminthic proteins due to the killed parasites, anaemia, malnutrition, and dehydration due to catharsis. Often other forms of therapy have to work in conjunction with chemotherapy, eg. the restoration of electrolyte balance, the treatment of anaemia, and the improvement of nutrition.
- 3) Many helminths have at least one stage of development in the intestinal tract. This makes it possible to administer enteric medications. It may be possible to diagnose helminthiasis by egg-count or parasite count in the stool.
- 4) Parasites usually have preferred sites of localization. The preparation of the medication and effective concentration should take the phenomenon of site selection into account.
- 5) Most parasites usually undergo several developmental changes in the host. Complex migrations often occur. The pathological stage is not necessarily the gastro-intestinal stage although the parasite may mature there.

The above generalizations have to be considered in the development of anthelmintics.

The development of a potential new drug consists of three basic stages: the finding of a potential new drug, the preclinical and clinical evaluations.

There are different methods of approach to the determination of new drug candidates:

- 1) Serendipity or random trial and error could account for the discovery of several pharmacological compounds, especially prior to the advent of modern medicine. The male fern, Dryopteris filix-mas, used for the treatment of gastro-intestinal cestodes like Taenia saginata, Diphyllobothrium latum, and Hymenolepis nana, was probably discovered in this manner.
- 2) New uses could be found for old drugs. Piperazine, which is presently used against A. lumbricoides and Enterobius vermicularis, was used in the early twentieth century for the treatment of gout (Rollo, 1971).
- 3) The extraction of a natural regulator from an animal, eg. antisera, is well-developed in bacterial infections. This is, however, a young and developing field in helminth research. There are many inherent difficulties since helminths are complex metazoans and the immune response could involve an intricate interplay of the humoral, cell-mediated, and inflammatory responses.
- 4) The empirical screening of chemicals, using in vivo and in vitro methods, has resulted in the discovery of several anthelmintics. This is further elaborated in Part II : Section C. This is, presently, the most common method used.
- 5) The deliberate modification of an active agent has resulted in the development of thiabendazole, a benzimidazole compound, which has a wide anthelmintic range, and stibocaptate (antimony sodium dimer-captosuccinate), the most effective trivalent antimony antischistosomal compound which is not more toxic than the others.
- 6) The rational approach involves the logical formulation of a new chemical based on knowledge of parasite and host biochemistry and

physiology. This is advocated by Standen (1963) but Campbell (1978) argued against it on the following grounds. The rational approach is difficult because the state of the knowledge of the biochemistry and physiology of helminths is not as developed as that of the vertebrates and bacteria. Furthermore, adverse interacting effects often cannot be predicted.

In primary screening, it is possible to determine the quantity of the drug used by weighing, if pure chemical compounds are used. However, extracts of plants or animals are often used in primary screening. The quantity, thus, has to be determined by the biological response. Bioassay can thus, be defined as "the estimation of drug potency by reactions of living organisms or their components" (Jenden, 1976). In the design of bioassays, a reference standard is needed. In order to minimize variation in the host, factors such as age, sex, animal strain, weight, and environmental conditions should be standardized. The biological response should be objectively measured; preferably, a double-blind study is recommended.

This project is concerned with primary screening which takes into account the easy availability of the host-parasite model, economics, and dose analogy of the laboratory animal models to the target species. It is, of course, accepted that results applicable to a laboratory model are not necessarily directly transferable to the human-parasite situation as reflected in

'Know then thyself, presume not God to see,

The proper study of mankind is man'.

- Alexander Pope (An Essay on Man)

A good laboratory animal-helminth model should include :

- 1) A host animal that is small and readily available,
- 2) A uniform infection that is easily established in the experimental animals,
- 3) Helminths with a short life-cycle that are easy to maintain in the laboratory (Theodorides, 1976).

Tables 3 and 4 list the primary screening organisms most frequently used in parasitological chemotherapeutic research.

A toxicity study must include a dose-response curve, which is a graphic picture of biological variation. It usually takes the form of a sigmoid curve. When comparing chemotherapeutic toxicity, internationally accepted standards, eg. ED_{50} and LD_{50} , have been developed. The rate of change in response due to the increase in dosage is usually greatest at the point where 50% of the population responds. This dose is conventionally referred to as the ED_{50} (effective dose for 50%) or median effective dose. If the effect is lethal for 50% of the population, the dose is called LD_{50} . The ratio of $LD_{50} : ED_{50}$ is defined as the therapeutic index, which is a directly proportional measurement of the margin of safety.

When doubts concerning the validity of the primary screen arise, or when the chemotherapeutic substances indicate anthelmintic potential, a secondary screen is run. A secondary screen may therefore, be expected to demonstrate whether the activity extends to related parasites in different hosts and to indicate more clearly the compounds with the highest therapeutic index (Standen, 1963).

Metabolic studies are carried out on the promising drugs. A

Table 3: Experimental models in antiparasitic chemotherapy, nematodes¹

INFECTION	AGENTS	PREFERRED METHOD OF INFECTION	PREFERRED NONHUMAN HOST	REFERENCE DRUG (S)	CRITERIA USED IN EVALUATING DRUG EFFICACY	GENERAL ASSESSMENT OF MODEL	ACTIVITY PREDICTIVE FOR HUMAN PARASITES
Oxyuriasis or Enterobiasis.	<u>Syphacia obvelata</u> ; <u>Aspiculuris tetraptera</u> .	Natural infection with both species; can be facilitated by gang-caging of pathogen-free and infected animals.	Male mouse.	Pyrantel pamoate.	Compare (a) time course of ova shedding and (b) worm load upon necropsy in drug-treated and untreated animals.	Good correlation to higher species; inexpensive model.	<u>Enterobius vermicularis</u> .
Ancylostomo-	<u>Ancylostoma caninum</u> ; <u>Nippostrongylus brasiliensis</u> ; <u>Heligmosomoides dubius</u> .	Administration by subcutaneous injection or by gavage of filariform larvae.	Dog; Rat; Mouse.	Pyrantel pamoate or bephenium.	Compare load by examining intestinal mucosal scrapings upon necropsy in drug-treated and untreated animals.	Excellent model with high predictive potential	<u>Ancylostoma duodenale</u> ; <u>Necator americanus</u> . (cont.)

1. Prepared by H.H. Gadebusch (1976), modified by L.Chan, using data from Standen (1963).

Table 3 (cont.)

INFECTION	AGENTS	PREFERRED METHOD OF INFECTION	PREFERRED NONHUMAN HOST	REFERENCE DRUG (S)	CRITERIA USED IN EVALUATING DRUG EFFICACY	GENERAL ASSESSMENT OF MODEL	ACTIVITY PREDICTIVE FOR HUMAN PARASITES
Ascariasis.	<u>Ascaridia galli</u> ;	Adminis- tration by gavage or in the feed of unhatched ova con- taining rhabditi- form lar- vae.	Chicken;	Pipera- zine or pyrantel pamoate	Compare (a) time course of ova shed- ding and/or (b) worm load upon necropsy in drug-treated and untreated animals.	Relatively inexpensive primary screening model.	<u>Ascaris lumbricoides</u> ;
	<u>Toxocara cati</u> ;		Mature cat;			Careful evaluation of drug efficacy cannot be over em- phasised; direct quantitative extrapolation to man often is not possible.	
	<u>Toxocara canis</u> .		Mature dog.				(cont.)

Table 3 (cont.)

INFECTION	AGENTS	PREFERRED METHOD OF INFECTION	PREFERRED NONHUMAN HOST	REFERENCE DRUG (S)	CRITERIA USED IN EVALUATING DRUG EFFICACY	GENERAL ASSESSMENT OF MODEL	ACTIVITY PREDICTIVE FOR HUMAN PARASITES
Trichinosis or Trichinellosis.	<u>Trichinella spiralis.</u>	(a) Feeding of rat or mouse muscle containing infective larvae or (b) administration by gavage of isolated larvae infection.	Rat; Mouse.	Thiabendazole.	Compare worm in striated muscle and intestinal mucosa upon necropsy, in drug-treated and untreated animals.	Model adequate but careful study of time course of infection is mandatory.	<u>Trichinella spiralis.</u>
Strongyloidiasis.	<u>Strongyloides ratti.</u>	Facilitate percutaneous invasion by bathing animals in water containing the filariform larvae.	Rat.	Thiabendazole.	Compare worm load by examining intestinal mucosal scrapings upon necropsy in drug-treated and untreated animals.	Excellent inexpensive model, high predictive potential.	<u>Strongyloides stercoralis.</u>

(cont.)

Table 3 (cont.)

INFECTION	AGENTS	PREFERRED METHOD OF INFECTION	PREFERRED NONHUMAN HOST	REFERENCE DRUG (S)	CRITERIA USED IN EVALUATING EFFICACY	GENERAL ASSESSMENT OF MODEL	ACTIVITY PREDICTIVE FOR HUMAN PARASITES
Trichuriasis	<u>Trichuris vulpis</u> ; <u>Trichuris muris</u> .	(a) Administration by gavage of unhatched ova containing larvae or (b) natural infection.	Dog; Mouse.	Mebendazole; Thiabendazole.	Compare the course of shedding of ova and adult worms in stool, drug-treated and untreated animals.	Extrapolation to man is excellent.	<u>Trichuris trichiura</u> .
Dracontiasis	<u>Dracunculus medinensis</u> .	(a) Ingestion from water of <u>Cyclops</u> containing third-stage larvae; (b) natural infection.	Dog	Thiabendazole; Niridazole.	Compare worm load upon necropsy in drug-treated and untreated animals.		<u>Dracunculus medinensis</u> .
Filariasis	<u>Litomosoides carinii</u> .	(a) Invasion via the bite of the rat mite (<u>Bdellonyssus bacoti</u>) harbouring the infective larvae; (b) natural infection.	Immature cotton rat (<u>Sigmodon hispidus</u>); Immature white rat	Diethylcarbamazine.	Compare microfilaremia in blood as a function of time after last drug dose in drug-treated and untreated animals.	Most practical model for filaricidal substances.	<u>Wuchereria bancrofti</u> ; <u>Brugia malayi</u> ; <u>Onchocerca volvulus</u> ; <u>Acanthocheilonema perstans</u> ; <u>A. streptocerca</u> ; <u>Mansonella ozzardi</u> ; <u>Loa loa</u> .

Table 4: Experimental models in antiparasitic chemotherapy, cestodes¹ .

INFECTION	AGENTS	PREFERRED METHOD OF INFECTION	PREFERRED NONHUMAN HOST	REFERENCE DRUG (S)	CRITERIA USED IN EVALUATING DRUG EFFICACY	GENERAL ASSESSMENT OF MODEL	ACTIVITY PREDICTIVE FOR HUMAN PARASITES
Hymenolepis- sis.	<u>Hymenolepis nana</u> ;	Administra- tion by gavage of ova from gravid pro- glottids of the mature worm or feeding of cysticeroid- infected beetles (Tenebrion- idae).	Mouse;	Niclos- amide	Compare worm load (small intestine in parasitized, drug-treated and untreated animals; definitive evaluation must be based on removal of all scolices.	Generally poor models but calcu- lation of chemothera- peutic index using large numbers of animals is possible; dose-response data are difficult to obtain.	<u>Hymenolepis nana</u> ; <u>Diphylliobothrium</u> <u>latum</u> .
	<u>Hymenolepis</u> <u>diminuta</u> .	Feeding of Rat. cysticeroid- infected beetles (Tenebrion- idae) espe- cially <u>Tribolium</u> <u>confusum</u> or <u>Dermestes</u> <u>lardarius</u> .					

(cont.)

Table 4 (cont.)

INFECTION AGENTS	PREFERRED METHOD OF INFECTION	PREFERRED NONHUMAN HOST	REFERENCE DRUG (S)	CRITERIA USED IN EVALUATING DRUG EFFICACY	GENERAL ASSESSMENT OF MODEL	ACTIVITY PREDICTIVE FOR HUMAN PARASITES
Taeniasis <u>Taenia taeniaeformis</u> ;	Administration by gavage of larvae (<u>Cysticercus fasciolaris</u>) obtained from rat liver.	Cat;	Niclosamide	Compare worm load (small intestine) in parasitized, drug-treated and untreated animals; definitive evaluation must be based on removal of all scolices.	Good model; adult worms are usually eliminated more easily in the cat than in man.	<u>Taenia solium</u> ; <u>Taenia saginata</u> ;
<u>Hymenolepis nana</u> ; <u>Hymenolepis diminuta</u> ;	As in hymenolepis.	Mouse; Rat;				
<u>Cysticercus fasciolaris</u> .	Administration by gavage of ova obtained from gravid proglottids of the mature worm (cat small intestines).	25-day old male rat.	None	Compare viable strobilocerci load in liver in drug-treated and untreated animals.		<u>T. solium</u> (cysticercosis).

study of the absorption, distribution, and excretion of the drug will determine the quantity of drug that reaches the site of action and the length of time available for the exertion of pharmacological action. The metabolism of the drug is also important since the metabolic products often determine the activity of the compound. A study of the side effects is also important. This is implicated in piperazine, which may produce or exacerbate epileptic seizures in predisposed patients. It is thus, contraindicated in epileptic patients (Botero, 1978).

Chemicals which survive the preclinical testing and toxicity evaluation, must be evaluated on the target species, which is Homo sapiens, in the present consideration. Although intensive preclinical testings may have been done, the importance of species differences in understanding the mechanism of the action of many drugs, has become increasingly evident (Hucker, 1970). Interspecific differences in drug responsiveness may be caused by variations in the fate of the drug (pharmacokinetic variations), i.e. its absorption, distribution, excretion, and biotransformation, or caused by variations of the responding systems (pharmacodynamic variations), or effected by both. This could apply to both the host's and parasite's systems. Strain differences generally involve the same mechanism as species differences. However, interspecific and interstrain similarities, analogies and correlations also exist. These similarities provide the basis for preclinical evaluation of candidate drugs (Jacob, 1968). The extrapolation of experimental results from animals to humans should be done with caution. Bioethics play an essential role in clinical evaluation. The code laid down by the Nuremburg judges sets the guidelines of the ethical principles governing drug investigation in humans.

Section C : A résumé of *in vivo* and *in vitro* experiments in Parasitology.

Two types of *in vivo* tests commonly used today are the critical test and the controlled test. In the critical test, the parasitized animal is administered with a suitable dose of the test compound. After the administration of the anthelmintic and several days later, all the faeces passed are examined for the presence of worms. The animals are killed at the end of the experiment and the gastro-intestinal tracts are processed to enumerate the surviving worms. The percentage efficacy is calculated using the following formula:

$$\frac{\text{Parasites eliminated in faeces}}{\text{Parasites eliminated in faeces} + \text{Parasites surviving}} \times 100$$

(Gibson, 1963; Leiper, 1952; Standen, 1963; Steward, 1955; Theodorides, 1976). This method is well-suited for animals with short intestines which harbour large helminths which are not easily digested.

Hall (1921) discovered the anthelmintic activity of carbon tetrachloride with this method. Despite the limitations of this method, it became the basis of many screening procedures prior to 1970.

Two groups of animals, naturally or artificially infected, are used in the controlled test. One group is treated while the other serves as the untreated control. A few days after the administration of the anthelmintic, all the animals are killed and processed to recover the parasites. The percentage efficacy of the worms removed is estimated by this formula:

$$\frac{\text{Parasites in control group} - \text{Parasites in treated animals}}{\text{Parasites in control group}} \times 100$$

(Baker, 1963; de Carneri, Cavier, Erhardt, Friedheim, Hawking, and Vita, 1973; Theodorides, 1976). This test is suitable for any host or organ parasitized. The combined use of the laboratory animal-helminth system with the controlled test, resulted in an unparalleled advance in the field of helminth chemotherapy. The critical test is, however, more popular when large animal-helminth systems are used (Gibson, 1963).

There are many variations to the laboratory animal-helminth model theme. An exposition of the historical evolution of this system is attempted.

Theodorides (1976) noted that Swartz and Porter (1937) artificially infected rats with larvae of Nippostrongylus brasiliensis, treated them with the test compounds orally and evaluated their efficacy with the critical test as they were disillusioned with the inadequacy of in vitro tests on earthworms or leeches to predict anthelmintic activity. Whitlock (1943) and later Rogers (1944) applied statistical analyses to improve accuracy. The controlled test was used by them. Whitlock and Bliss (1943) introduced a bioassay technique for anthelmintics involving rigid statistical analyses. Brackett and Bliznick (1949) worked with Nippostrongylus muris (= brasiliensis) in mice, mixing the compounds with the feed and using the controlled test.

In oxyuriasis, Hsieh (1952) and Thompson and Reinertson (1952) preferred the artificial inoculation of 500 infective eggs of Aspicularis tetraptera into mice to the natural infection of Syphacia obvelata used by Chan (1952), Brown, Chan, and Ferrell (1954), Lynch and Hoegl (1959), and Actor, Anderson, Dicullo, Ferlanto, Hoover, Pagano, Ravin, Scheidy, Stedman, and Theodorides (1967). The controlled test was used by the workers mentioned above.

Leiper (1952) used naturally-infected chickens with Heterakis gallinarum, Ascaridia galli, and Capillaria obsignata. The earlier experiments by Hewitt and Gumble (1957) on Nematospiroides dubius in mice was expanded by Lynch and Nelson (1959), who used more anthelmintic doses. Sheffield, Meisenhelder, and Thompson (1959) studied the effects of reference anthelmintics against N. dubius and pinworms in mice. de Carneri et al. (1973) recommended the use of N. brasiliensis in rats and N. dubius in mice as laboratory animal-helminth models for hookworms (Ancylostoma duodenale and Necator americanus) of humans.

Hall and Foster (1918) reported some moderately successful cases of anticestodal treatment in poultry and dogs with the critical test using 1) simple purgatives (eg. castor oil, calomel, epsom salt), 2) a group of anthelmintics which share a mineral base with coal-tar products (eg. ether, phenols), and 3) a group covering the plant-derived anthelmintics (eg. Oleoresin aspidii, Areca catechu, pelletierine tannate). Theodorides (1976) then traced the search for anticestodal compounds from the studies of Harwood and Jerstad, who in 1938, successfully produced infections of Raillietina cesticillus in chickens, to Holton, who about 10 years later, used mice infected with Hymenolepis nana and Hymenolepis diminuta. Extract of male fern (Dryopteris filix-mas) was used as a positive control. The drug efficacy was assessed as the percentage mass of worms recovered from the faeces compared to the total mass of worms recovered (= weight of worms recovered from the faeces - weight of worms recovered from the killed mice). Steward (1955) artificially infected mice with the ova of H. nana since he realized that the age of the cestodes and thus the size varied greatly in natural infections. It was only in 1960 that the microscopic examination of

the small intestine for the presence of scolices was widely used as a method for the evaluation of anticestodal compounds.

Howes and Lynch (1967) and Brody and Elward (1971) are among those who used various permutations of the 4 helminth systems (A. tetra-
ptera, S. obvelata, N. dubius and N. brasiliensis) in mice. The test compounds were given by gavage, in medicated feeds, or both. The importance of particle size of the test compounds was noted by them.

The trend of screening and evaluation procedures seems to be towards using small laboratory animals like mice and rats and a few "good" helminth models, like N. brasiliensis, N. dubius (= Heligmosomoides dubius), H. nana, H. diminuta, A. tetra-
ptera, S. obvelata, Trichinella spiralis and Trichuris vulpis, for the primary screen. Rigid statistical analyses and the controlled test are used.

There is no doubt that "the final test of the effectiveness of an anthelmintic is its administration to the host and the determination of the percentage of the parasites expelled" (Lamson and Brown, 1936). However, when it is difficult to find a convenient laboratory host animal, in vitro tests would have to suffice although they are more appropriate for discovering specific activity. It has often been argued that a parasite removed from the host is in an abnormal environment and is actually a dying organism (Leiper, 1963). There have been attempts to rectify this situation by in vitro cultures of helminths (Hopkins, 1967; Taylor and Baker, 1968).

To study the effect of drugs on earthworms or leeches as an in vitro evaluation of anthelmintic activity is outdated (Leiper, 1952). As this did not prove satisfactory, various workers introduced alternatives. Baldwin (1943) attached a ligatured fragment of A. lumbricoides

to a recording lever, immersed in a balanced saline medium at about 38°C - 39°C, simulating as closely as possible the natural host conditions. With this kymographic method, he tested the effect of a total of fifty-eight compounds. He recommended this method for the detection of anthelmintic potency as the procedure did not require exceptional technical skill, is relatively economical concerning drugs and living materials used, and is rapid and convenient in application. However, the results tended to be variable. The method was elaborated further by Baldwin and Moyle (1947; 1949).

To avoid damaging the parasites and to retain the normal conditions as closely as possible, Rogers (1944) perfused a test solution through the intestinal loop of a rat experimentally infected with N. muris (= brasiliensis). After a time period, the intestinal segment was opened. The number of dead and living parasites was counted.

Kerr and Cavett (1952) adapted the graphic recording method to whole intact nematodes like A. galli.

Goodwin (1958) inserted whole intact pig ascarids into cylinders made of nylon stockings. They were suspended in physiological saline at 37°C. The displacements of the cylinder caused by the worm's movements were recorded. With this method, the parasites' cuticles were not damaged and their digestive tracts were unimpaired by ligatures.

The observation of the rhythmic contractions in A. lumbricoides which can be recorded in a kymograph and have been used for the assay of anthelmintics (Baldwin, 1943; Baldwin and Moyle, 1947; Baldwin and Moyle, 1949; Goodwin, 1958; Kerr and Cavett, 1952) and the "spontaneous electrical activity" (Jarman, 1959) prompted De Bell, Del Castillo, and Sanchez (1963) to study the properties and characteristics of these

potentials. The electrical and mechanical activity of the oesophageal cells of A. lumbricoides were recorded and correlated by Del Castillo and Morales (1967). The works mentioned above are considered not merely as an increase in biological knowledge which would help in the study of anthelmintics but also as tools for the exploration of interesting types of invertebrate organisms (Del Castillo, 1969). A. lumbricoides is rapidly becoming an useful laboratory animal for the investigation of some basic problems in biology like the interplay between excitatory and inhibitory synaptic influences, and the action of drugs on the frequency and amplitude of myogenic action potentials (Del Castillo and Morales, 1969). The in vitro studies on A. lumbricoides originally evolved as an applied technique for testing anthelmintic efficacy but in recent years, the extrapolation of the results to more general biological problems is increasing. Two useful references in electrophysiological techniques are Bures (1967) and Nastuk (1964).

The in vitro studies on H. diminuta are not as varied as those described above for the nematodes. Sen and Hawking (1960) noted the concentration of the compounds that killed H. nana, by immersing it in vitro in a media broth with test drugs for 24 hours. The most active compounds killed at a concentration of 10^{-6} or less. Oil of chenopodium, extract of cashew nut, dichlorophen, and tartar emetic were in this group. Male fern extract, arecoline, hexylresorcinol, and quinacrine killed at a concentration of 10^{-5} .

Duguid and Heathcote (1950a; 1950b) connected a length of Moniezia expansa suspended in Tyrode solution at 39°C - 40°C to a kymograph lever and measured the response to drugs.

In vivo and in vitro studies should complement each other.

In vitro tests should ideally be designed to discover more specific activity or to support the in vivo results but when the screening of hundreds of substances is involved, it would be more economical and feasible to use in vitro methods. When the in vitro results warrant further study, in vivo methods could be used. Positive in vivo results could be supplemented by in vitro experiments aimed at elucidating the mode of action.

Section D : A literature review of pyrantel pamoate, niclosamide, piperazine, and arecoline.

An ideal anthelmintic should have the widest possible therapeutic index, which is the difference between LD_{50} and ED_{50} , and yet does not interrupt the life of the host or retard its development. It should also be economical since the target consumers are most likely to be from the poorer, developing countries.

Pyrantel pamoate (Combantrin^R) and piperazine citrate (Antepar^R) were the reference drugs used for my experiments on nematodes, while niclosamide (Yomesan^R) and arecoline hydrobromide were used for my experiments on cestodes. Pyrantel pamoate, piperazine citrate, and niclosamide were selected for the laboratory evaluation because they are considered^o the drugs of choice for most nematode and cestode infections (Botero, 1978).

Pyrantel pamoate

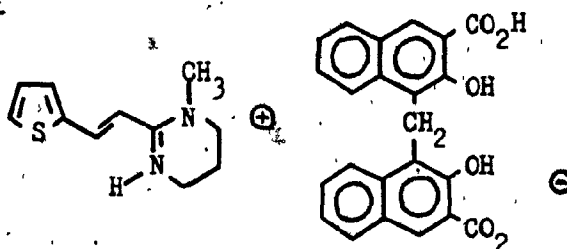


Figure 2. Structural formula of pyrantel pamoate (Pitts, N.E. and Migliardi, J.R. (1974) Antiminth (pyrantel pamoate) : The clinical evaluation of a new broad-spectrum anthelmintic. Clinical Pediatrics 13: 87-94)

Pyrantel pamoate is selected for the treatment of Ascaris lumbricoides and Enterobius vermicularis in the Drug of Choice 1974 - 1975 (Kesh and Hopkins, 1974), Current Therapy 1974 (Conn, 1974), and

also of hookworm (Botero, 1978), although it has yet to be officially legalized in USA as an anthelmintic against hookworm infections.

Its chemical name is trans - 1,4,5,6 - tetrahydro - 1 - methyl - 2 - (2 - thienyl) vinyl - pyrimidinehydrogen pamoate. It is insoluble in water, tasteless, and safe. It is marketed as Antiminth^R and Combantrin^R. The dosage recommended (Ferreira, 1974) is a single dose of up to 50 mg/(kg body weight) or 5 mg/(lb body weight)/day (which is equivalent to 11 mg/(kg body weight)/day).

The pyrantel group which was first extensively worked on by Austin, Courtney, Danilewicz, Morgan, Conover, Howes, Lynch, McFarland, Cornwell, and Theodorides (1966) is probably the new broad-spectrum anthelmintic of choice (Ferreira, 1974). High overall efficiency against E. vermicularis (97.2%) and A. lumbricoides (97.5%) had been shown using just single doses of 5 mg/(lb body weight)/(base activity) (Pitts and Migliardi, 1974). Pyrantel affects several cholinergic mechanisms (Aubry, Cowell, Davey, and Shevde, 1970; Eyre, 1970). The metabolism of pyrantel in 5 species (sheep, ox, pig, dog, and rat) was studied by Faulkner, Figdor, Monroe, von Wittenan, Stopler, and Wood (1972). Many clinical trials indicate that pyrantel pamoate is efficacious with a cure rate of at least 76%, and often 100% is well-tolerated (Bell and Nassif, 1971; Botero and Castano, 1973; Cervoni and Oliver-González, 1971; Desowitz, Bell, Williams, Cardines, and Tamarna, 1970). The molecular basis of the anthelmintic action of pyrantel is partially unravelled (Van den Bossche, 1976a).

Piperazine

The introduction of piperazine and its salt for the treatment.

of A. lumbricoides and E. vermicularis had been adjudged by Saz and Bueding (1966) as one of the most significant advances in helminth chemotherapy. Piperazine citrate was considered to be the drug of choice for ascariasis and enterobiasis (Brown, 1969; Davis, 1973; Elslager and Thompson, 1962; Kean and Hopkins, 1974; Pratt, 1973; Rollo, 1971), prior to 1974.

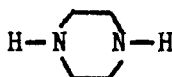


Figure 3. Structural formula of piperazine (base).

Its chemical name is diethylenediamine. Its simple salts - piperazine adipate, citrate, phosphate, sulphate, tartrate, and hydrochloride - are more stable than the piperazine base. The dosage is customarily expressed in terms of the hydrate equivalent. The hexahydrate contains 44% of the base. 100 mg of piperazine hydrate is approximately equivalent to 125 mg of piperazine citrate, to 120 mg of piperazine adipate, and to 104 mg of piperazine phosphate. The salts are white crystalline powder with a saline taste and are readily soluble in water except for the insoluble phosphate and the adipate.

Piperazine has been in use for over 30 years (Botero, 1978). It is the most commonly used and is one of the few that are practical for mass treatments as indicated by the WHO Expert Committee on the Control of Ascariasis, 1967 (Davis, 1973).

The mechanisms of the paralyzing action of piperazine on A. lumbricoides muscle was investigated extensively by Del Castillo, de Mello, and Morales (1964). Recently, its mechanism of action was

elucidated. A blocking agent of acetylcholine in the myoneural junctions of the helminths produces a flaccid paralysis which facilitates the elimination of the worms by normal intestinal peristalsis. However, piperazine has only a minute blocking effect on the myoneural mammalian junctions. The side effects are varied.

Niclosamide

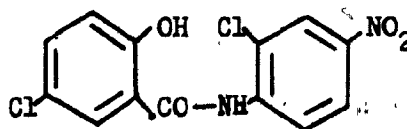


Figure 4. Structural formula of niclosamide.

Its chemical name is N - (2' - chloro - 4' - nitrophenyl) - 5 - chlorosalicylamide. Its molecular formula is $C_{13}H_8Cl_2N_2O_4$ and its molecular weight is 327.1. It is yellowish-white, tasteless, and odourless, and it is insoluble in water.

Its trademark is Yomesan^R. The dosage schedule is :-

For beef (Taenia saginata), pork (Taenia solium), and fish (Diphyllobothrium latum) tapeworms :-

Adults and children above 6 years 4 tablets.

Children between 2 and 6 years..... 2 tablets.

For the dwarf tapeworm (Hymenolepis nana) :-

Adults and children above 6 years 4 tablets on the first day, then 2 tablets daily for 6 days.

Children between 2 and 6 years..... 2 tablets on the first day, then 1 tablet

500 mg per tablet (Boehringer Ingelheim, 1974) daily for 6 days.

Niclosamide represents a major advance in the treatment of cestodiasis (Davis, 1973). Even at low concentrations, it has an inhibitory effect on ATP synthesis in the mitochondrion of Hymenolepis diminuta (Pratt, 1973; Saz, 1972; Van den Bossche, 1972). It also has a high chemotherapeutic index. The low toxicity of niclosamide could be due to its inability to penetrate the host tissue (Van den Bossche, 1976b).

Numerous trials have indicated that niclosamide has a high curative efficacy with T. saginata, T. solium, D. latum, and H. nana.

Arecoline

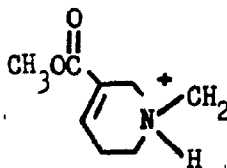


Figure 5. Structural formula of arecoline base.

Its molecular formula is $C_8H_{13}O_2N$. It is one of the older anticestodals of natural origin which is still used today in veterinary medicine, mainly in dogs. It is an alkaloid extracted from the seeds of the betel nut palm (Areca catechu). It is rather unstable but its derivatives (arecoline hydrobromide, arecoline-acetorsol, and arecoline carboxyphenylstilborate) have a longer shelf life.

Arecoline hydrobromide, $C_8H_{13}NO \cdot HBr$, N.F., is a fine, white, bitter, optically inactive, crystalline substance, which has a melting point of $169^\circ - 171^\circ C$. It is relatively stable both when dry and when in an aqueous solution. One gram of it dissolves in 1 ml of water and 10 ml of alcohol. It should be stored in a light-resistant container.

Arecoline has a muscarino-mimetic action on cholinergic effector cells of glands, smooth muscles, and myocardia of mammals. Consequently, the parasympathomimetic effects stimulate secretion of the glands of the digestive tract and increase peristaltic movements of the gut.

The anticestodal effect of arecoline is facilitated by the combination of its temporary paralytic action on the worm. Consequently, the cestodes are detached from the intestinal mucosa. The simultaneous stimulation of increased peristalsis assists in the expulsion of the tapeworm (Roberson, 1977).

It is generally not recommended for human use. For general use in dogs, a dosage of approximately 1 mg/(kg body weight) is suggested (Batham, 1946).

Section E: Notes on the pharmacognosy of the herbs tested.

The botany of the traditional Chinese herbal anthelmintics selected for the laboratory evaluation will be outlined below. Most of the information given is liberally taken from Keys (1976) and the Merck Index (1960).

Areca catechu

檳榔

Family : Palmae

It is commonly known as the betel palm. It ranges from 10 - 30 m high with 10 - 12 leaves constantly forming the head. The leaves are 1.0 - 1.8 m long, are pinnate with the upper segments joined, with the petiole sheathed and with the sheath encircling the tip of the stipule. The inflorescence is paniculate, axillary, and monoecious. The female flowers are not numerous and they grow from the axis of the spadix, while the male flowers are numerous and very small, and are located at the tip of the branches. The fruit is a monospermous berry.

The seeds, known as betel nuts, are globular and hard. The interior is marbled brown and white. The taste is bitter and astringent. They contain fixed oil (palmitic, oleic, stearic, caproic, caprylic, lauric, myristic acids), mannosan and galactans, red tannin, choline and alkaloids (arecoline, arecolidine, arecaldine = arecaine, guvaccine, and guvacine).

It grows in Laos, Northern and Southern Vietnam, and Malaysia.

Cucurbita pepo

南瓜

Family : Cucurbitaceae

The pumpkin is an annual prostrate vine. Its stems and petioles are prickly. The leaves are triangular-oval in shape, have long

petioles and are prominently 5-lobed with acuminate tips. The flowers are large, yellow, and monoecious. The fruit is large, furrowed, and usually orange.

The seeds are yellowish or grayish white. They are thick and have a tapering tip. They are oval to elliptical in shape and the margin is entire. The surface is convex and smooth. The taste is sweet and oily. The seeds contain fixed oil, acrid resin, myosin, vitellin, and sugar.

It is cultivated worldwide.

Melia azedarach 苦楝

Family : Meliaceae

It is commonly known as the Chinaberry tree. The tree is 20 m tall. The leaves are alternate and pinnate. The leaflets have margins which are irregularly dentate. The flowers are odouriferous, elongate, and purple. The fruit is a glabrous drupe about 1 - 3 cm in diameter. The pericarp is fleshy while the endocarp is ligneous. The seeds are black and elliptical.

The root epidermis and fruit are medicinal. The bark of the tree contains tannin and the alkaloid margosine. The seeds contain 60% of a fatty oil which is comprised of stearic, palmitic, lauric, valerianic, and butyric acids.

It is cultivated in China, Indochina, India, Iran, Syria, Madagascar, Guyana, and Antilles.

Mylitta lapidescens 雷丸

Family : Polyporaceae

It is a subterranean fungus which apparently grows in associa-

tion with the roots of the bamboo. The surface of these small, irregular globules is finely reticulate while the interior is compact and granular, and of a brown or reddish colour. When dried, the fungus becomes small, hard, and shrivelled nodules of a brownish gray colour.

It has a bitter taste. It contains 3% of an odourless and tasteless substance which is soluble in water and glycerine but insoluble in ether and alcohol. It has been identified as a proteolytic enzyme, with vermifugal action, by Lu Kuei-Sheng in a Chinese journal (cited in Keys (1976)).

It is found in Northern China.

Quisqualis indica

使君子

Family : Combretaceae

The Rangoon creeper is a woody vine. Its leaves are opposite, oval, and the tips are briefly acuminate. The inflorescence is a compact terminal spike. The fruit is a dry 5-sided capsule which is chestnut brown in colour, leathery, monospermous, and dehiscent. The oily seed is enclosed in a thin and brittle pericarp. The seeds contain 25% fatty oil comprising of oleic, myristic, palmitic, stearic, and linoleic acids, gum and resin.

It is cultivated in India, Malaysia, Vietnam, Laos, and the Philippines.

Ulmus macrocarpa

糖菓

Family : Ulmaceae

The tree is 10 m tall. The leaves are alternate, obovoid, or elliptical; the margins are doubly toothed, and the petioles are pubescent. The flowers are polygamous and apetalous.

The fruit is an obovoid samara. The taste and odour are strong and pungent.

Methods of identifying powdered vegetable drugs are described by Jackson and Snowden (1968). The Indian Council of Medical Research (1976) published a useful work titled Medicinal Plants of India (Satyavati, Raina, and Sharma, 1976), which gives the various common names of the plants used, the medicinal properties, and the chemistry and pharmacology of the active ingredients.

PART III

Introduction

Part I provided the theoretical foundation on which this laboratory evaluation was based. The selection of the herbs was based on Table 2. The water extract was used since this is the form commonly taken by the Chinese (Li, 1974). If the herbs were found to significantly reduce the adult worm load, a qualitative analysis of the herbs would then be appropriate.

The host-parasite models were selected from Tables 3 and 4. Nippostrongylus brasiliensis in the Sprague-Dawley white rat was used as a hookworm model since it is an excellent laboratory model with a highly predictive potential. Although Taenia taeniaeformis in cats is a better laboratory model for Taenia solium and Taenia saginata, Hymenolepis diminuta was used as it was easily available, it can also infect humans, and the rat is preferred to the cat as a laboratory host. Ascaris lumbricoides was selected as an in vitro model since it is large, easily available, and infective to humans. The criteria of cure will be dealt with in the individual sections.

On the assumption that the traditional Chinese herbal anthelmintics selected are efficacious, significant reductions in the recovery of adult worms in the treated animals should be detected when compared with that of the untreated controls. If the reductions are found not to be significant, it would imply that the herbs are not anthelmintic in the host-parasite model selected by the criteria and in the constraints defined. A reference drug was used to ensure that the rat-parasite model was analogous to the human-parasite relationship.

The working hypotheses set were that the decoctions of

- 1) Areca catechu and Cucurbita pepo,
Melia azedarach (seeds and roots),
Mylitta lapidescens, and
Quisqualis indica

would reduce the number of adult N. brasiliensis in the rat intestine as compared with that recovered from the untreated control, if they were anthelmintics,

- 2) A. catechu and C. pepo, and
Ulmus macrocarpa

would reduce the number of adult H. diminuta in the rat intestine as compared with that recovered from the untreated control, if they were anthelmintics,

and

- 3) The movement of A. lumbricoides would be affected by decoctions of Q. indica, if it were an anthelmintic.

I) In vivo experiments

General materials and methods

Male Sprague-Dawley albino rats from Canadian Breeding Farm and Laboratories Ltd., St. Constant, Québec, were used as the laboratory host of N. brasiliensis and H. diminuta. The rats were fed Purina rat chow ad lib. throughout. They were housed individually in cages with dimensions 19.5 cm by 24.5 cm by 18.0 cm.

The traditional Chinese herbal anthelmintics (abbreviated as TCHAs) used, A. catechu, C. pepo, M. azedarach, M. lapidescens, Q. indica, and U. macrocarpa (Plate 1), were purchased from Sterling Company, 283, Dundas Street West, Toronto, Ontario (Plate 2). They were selected on the basis of the frequency of mention in a number of Chinese anthelmintic prescriptions surveyed (Table 2) and their availability.

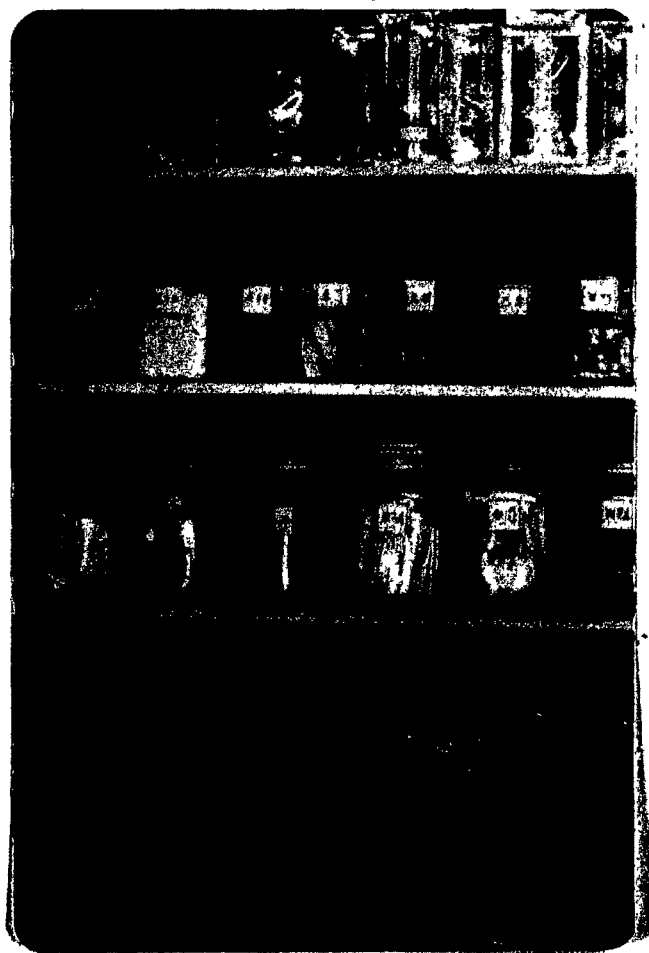
- A) The evaluation of the chemotherapeutic efficacy of A. catechu and C. pepo, M. azedarach, M. lapidescens, Q. indica, and pyrantel pamoate against adult N. brasiliensis in rats.

General materials and methods

The Institute of Parasitology strain of N. brasiliensis was used. Faecal pellets were collected from rats, 8 days post-infection. A 1:1 mixture of activated charcoal and faecal matter was made. The homogenized paste was put on a filter paper (Whatman No. 1) and placed above a layer of vermiculite which was saturated with water. These



Plate 2: Traditional Chinese medicine sold in a shop in
Toronto, Ontario.



coprocultures were incubated at 25°C. After 5 days, the infective larvae were recovered, using the Baermann funnel. The infective larvae were suspended in water at about 29°C, which activated the larvae, to facilitate rapid recovery.

1) Experiment 1

(a) Rationale:

The object of this preliminary experiment was to test as many of the available TCHAs as possible. A mixture of M. azedarach, M. lapidescens, and Q. indica was used since they were reputed for their anti-nematodal properties. Pyrantel pamoate was used as the Western reference drug. The method of the preparation of TCHAs was investigated and experimented on.

(b) Experimental design:

Three individual treatment groups: an untreated control group, a group treated with a mixture of M. azedarach, M. lapidescens, and Q. indica, and a group treated with pyrantel pamoate, were set up. Each had 5 replications (Table 5). The rats were randomly placed in each of the treatment groups.

(c) Materials and methods:

(i) Inoculation of the rats

The hosts were 4 week-old male Sprague-Dawley albino rats, of about 100 g. Each rat was infected with 200 ± 20 L₃, to simulate the moderate infection rate which occurs naturally. This low dose was

Table 5: Experiment 1 - Experimental design.

Treatment	Control	1:1:1 mixture of <u>M. azedarach</u> (seed), <u>M. lapidescens</u> , and <u>Q. indica</u>	Pyrantel pamoate
No. of replications (rats)	5	5	5

obtained by individual counting rather than by sampling, which is the method used with high infective doses. The inoculum was injected subcutaneously between the scapulae, using a 1 c.c. tuberculin syringe and 26G 3/8 Yale needle. The larvae which remained in the syringes and test-tubes were counted.

(ii) Preparation of a mixture of M. azedarach (seeds),

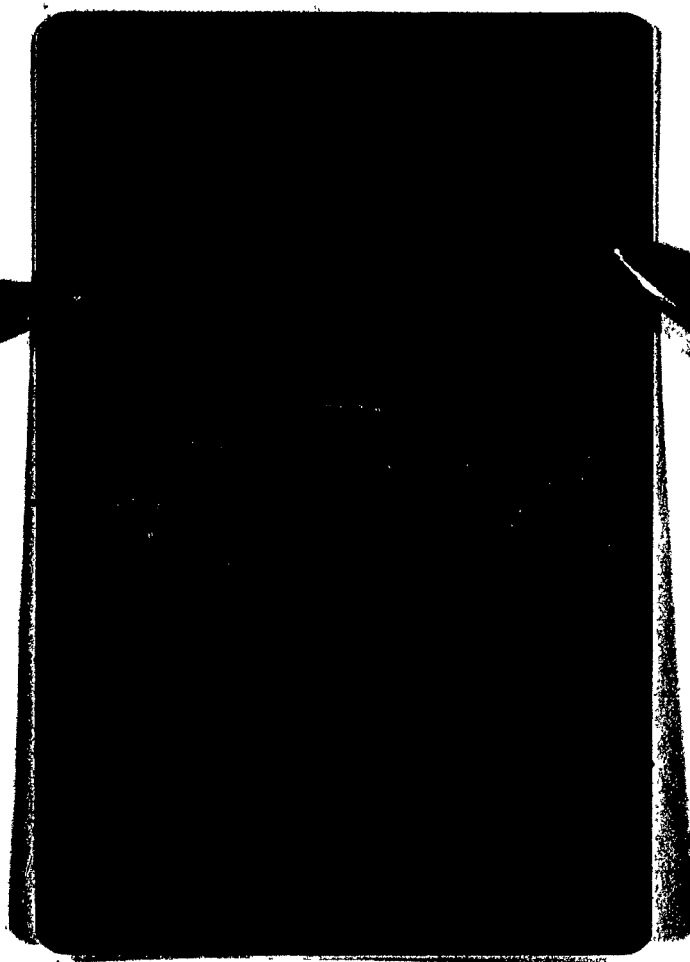
M. lapidescens, and Q. indica

M. azedarach seeds (3 ch'ien = 10.8 g ; Appendix 1), 10.8 g of M. lapidescens, and 10.8 g of Q. indica were pounded, mixed, and boiled in 900 ml distilled water for about 30 minutes to 720 ml in a clay pot (Plate 3). The mixture was gently heated for about 4 hours to concentrate to a level where 0.3 - 0.4 ml of the mixture could be fed to each of the rats daily. The concentration of the mixture was 1.62 g/ml (Appendix 2 - for dosage calculation). The preparation, refrigerated at 4°C, was used throughout Experiment 1.

(iii) Chemotherapeutic trials

The anthelmintics were fed on days 7, 8, and 9 post-infection at 16.00 hour after removing the food for 6 hours. The rats were lightly etherized if necessary. The controls were given 0.5 ml distilled water each. The pyrantel pamoate used (Appendix 3 - for dosage calculation) in all the experiments was commercial Combantrin^R, made by Pfizer Company Ltd., Montreal, Canada. The lot number was 702-18044. The anthelmintics and water were given intraoesophageally with a glass pipette with a blunted end. The dose of the herbal anthelmintics and pyrantel pamoate given was twelve times that recommended.

Plate 3: A clay pot used for the preparation of traditional Chinese herbal anthelmintics.



for humans and also on a weight, to weight ratio (Appendices 2 and 3).

(iv) Evaluation of the chemotherapeutic treatment

On the 10th. day post-infection, after fasting the animals for 6 hours, they were sacrificed by ether overdoses. The animals were sacrificed sequentially in 5 blocks of 3 animals, i.e. 1 animal from each treatment block. A mid-lateral incision was made and the stomach, small intestine, caecum, and large intestine, were removed. The small intestines were divided into 10 cm sections. They were placed in 0.85% saline and incubated in covered petri-dishes for an hour at 37°C. The number of adult N. brasiliensis recovered was counted.

(d) Results:

The % reduction of adult N. brasiliensis in rats is defined (Baker, 1963) as :

$$\frac{\text{Mean no. of parasites in control group} - \text{Mean no. of parasites in treated rats}}{\text{Mean no. of parasites in control group}} \times 100$$

The raw data and statistics for Experiment 1 are in Appendix 4. The results are tabulated in Table 6. The values for the groups treated with a mixture of herbs and pyrantel pamoate were 29.4% and 100% reduction of the worm load as compared with the control group, respectively (Figure 6). The effect of the chemotherapeutic treatments, as measured by the recovery of adult N. brasiliensis were tested using Analysis of Variance (ANOVA).

There was a highly significant ($P < 0.001$) added component due to treatment effects in the mean square among groups (treatment).

✓ Table 6: Experiment 1 - The mean number of adult N. bra-
siliensis recovered from the intestines of rats
treated with a 1:1:1 mixture of M. azedarach
(seed), M. lapidescens, and Q. indica and
pyrantel pamoate (± 95% confidence limits and
± standard error of the mean) and % efficacy
(refer to the text, pp. 107 for the formula).

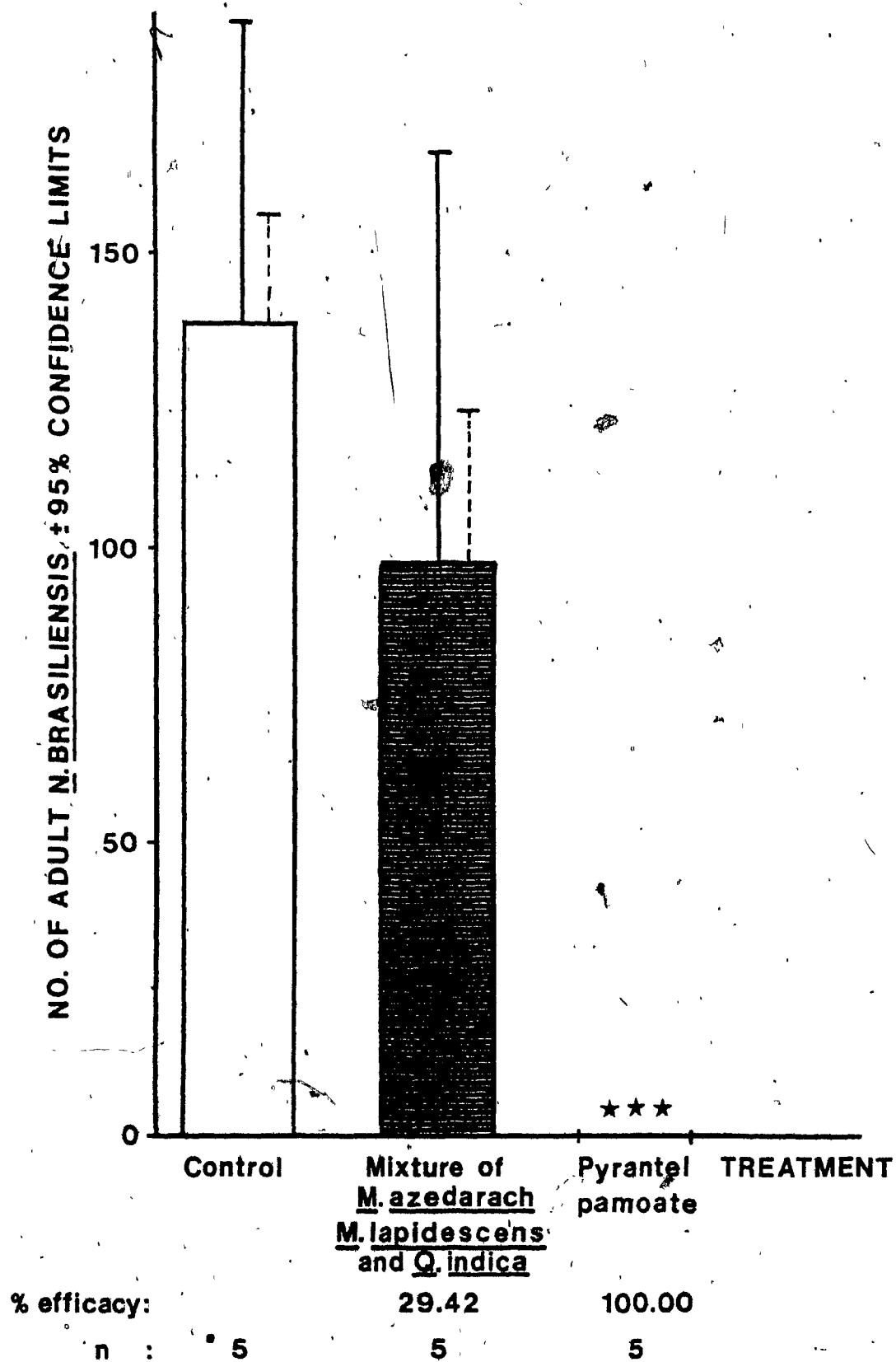
Treatment	Control	1:1:1 mixture of <u>M. azedarach</u> (seed), <u>M. lapidescens</u> , and <u>Q. indica</u>	Pyrantel pamoate
Mean no. of adult <u>N. brasiliensis</u> + 95% confidence limits + standard error N =	138.0 $\pm$ 50.7 138.0 $\pm$ 18.2 5	97.4 $\pm$ 69.9 97.4 $\pm$ 25.1 5	0.0 $\pm$ 0.0 0.0 $\pm$ 0.0 5
% efficacy	0.0	29.4	100.0

Figure 6: Experiment 1 - The effect of a 1:1:1 mixture of M. azedarach (seed), M. lapidescens, and Q. indica and pyrantel pamoate on the mean number of adult N. brasiliensis recovered from the intestines of rats $\pm$ 95% confidence limits (solid lines on the left) and $\pm$ standard error (dotted lines on the right).

*** = $P < 0.001$

** = $P < 0.01$

* = $P < 0.05$



The chemotherapeutic treatment had a significant effect on the number of adult N. brasiliensis recovered from the rats. The a priori and a posteriori tests revealed which of the chemotherapeutic treatments contributed to the significant difference.

The a priori tests gave the following results :

$$F_s = \frac{MS \text{ (control versus treatment)}}{MS_{\text{within}}} = 16.54^{**}$$

where MS = Mean squares = Sum of squares divided by the degree of freedom,

$$F_{.01} (1, 12) = 9.33 \quad \text{and}$$

$$F_{.001} (1, 12) = 18.60 \quad \text{and}$$

since $F_{.001} (1, 12) > F_s > F_{.01} (1, 12)$, the control is significantly different from the treatment groups at the 99% level.

$$F_s = \frac{MS \text{ (control versus herbal mixture)}}{MS_{\text{within}}} = 2.56$$

The control group was significantly different from the treatment groups but was not significantly different from the group treated with a herbal mixture since $F_s < F_{.05} (1, 12)$. It could be concluded that the effect of the herbal mixture was significantly different from that of pyrantel pamoate.

The Student-Newman-Keuls (SNK) test (Sokal and Rohlf, 1960) was the a posteriori comparison used. The a posteriori testing (Table 7) indicated that the results of the group treated with the herbal mixture was not significantly different from that of the control group. The results of the group treated with pyrantel pamoate was significantly

Table 7: A posteriori testing of the results from Experiment, using the Student-Newman-Keuls (SNK) procedure (From Sokal, R.R. and F.J. Rohlf. 1969. Biometry, pp. 239-246. San Francisco: W.H. Freeman and Company).

Treatment	Pyrantel pamoate 1:1:1 mixture of <u>M. azedarach</u> (seed), <u>M. lapidescens</u>, and <u>Q. indica</u> Control-
Mean no. of adult <u>N. brasiliensis</u> N = 5	0.0. 97.4 138.0

The underlined means are not significantly different ($P = 0.05$). This applies to all subsequent a posteorl testings.

different, at the 95% level, from that of the control group and that of the herbal mixture.

The Chinese medicine was boiled for several hours to concentrate it. It was possible that this could have reduced the activity of any active ingredients. The three herbs could also have had antagonistic effects.

2) Experiment 2

(a) Rationale:

The TCHAs were tested individually so as to avoid antagonistic or synergistic effects. Since the herbal mixture did not have a significant anthelmintic effect, the impact on 'health' was quantified. The rate of increase in weight and the amount of rat chow eaten (taken as a measure of appetite), were set as two criteria of 'health'. The preparation time of the herbs was reduced to minimize the risk of destroying the active ingredients. Pyrantel pamoate was again used as the reference drug.

(b) Experimental design:

Four treatment groups: control, a group treated with M. azedarach, one with Q. indica, and one with pyrantel pamoate, were used. These were subdivided into 2 subgroups, the infected and the uninfected. The infected treatment groups each had 4 replications while the uninfected groups had 2 replications each (Table 8). The uninfected animals were used as controls to measure the effect of chemotherapy without infection. Twenty-four rats were used in this experiment since this was the

Table 8: Experiment 2 - Experimental design.

Treatment	Control	<u>M. azedarach</u>	<u>Q. indica</u>	Pyrantel pamoate
No. of replications (rats infected with <u>N. brasiliensis</u>)	4	4	4	4
No. of replications (uninfected rats)	2	2	2	2

maximum number which could be handled so as to collect the data accurately.

(c) Materials and methods:

(i) Inoculation of the rats

The method was similar to that described in Experiment

1.

(ii) Quantification of the 'health' of the rats

The rats were weighed daily at 09.00 hour. Each rat was given 100 g of rat chow daily. The amount of rat chow consumed by the rat was calculated by the following formula :-

$$100 \text{ g} - (\text{Wt. of chow left} + \text{Wt. of chow scrappings left on the paper left below the cage}).$$

The rat chow consumed was weighed at 10.00 hour daily.

(iii) Preparation of the herbs

M. azedarach seeds (12 X 10.8 g = 129.6 g) were pounded, ground in the blender for 60 s (particles were filtered through a Fisher sieve mesh size 20) and boiled in 300 ml distilled water for approximately 20 minutes to 240 ml. Since the solution contained twelve times the dose of M. azedarach recommended for humans, the decoction did not require concentrating. Q. indica was prepared in a similar manner except the husks of the seeds of Q. indica were discarded. Only the seeds of Q. indica were pounded, ground, and used. The preparations were refrigerated at 4°C in sterilized bottles. The preparations were used throughout this experiment.

(iv) Chemotherapeutic trials

The rats were fasted for 6 hours prior to the chemotherapeutic treatments. Water was provided ad lib. throughout. The anthelmintics were fed on days 7, 8, and 9 post-infection. The chemotherapeutic regimen, dose, and method of feeding were described in Experiment 1.

(v) Evaluation of the chemotherapeutic treatment

The procedure was similar to that described in Experiment 1.

The % gain in weight was adopted as the measurement of the growth rate. The pretreatment growth rate of the rats in the various treatment groups had to be tested for homogeneity since the % gain in weight varies with age. The growth rate of the uninfected group was recorded as a control group for comparison.

The weight of rat chow eaten daily was taken as the measurement of appetite.

(d) Results:

The % gain in weight of the rats during a pretreatment period (day 6 to day 7) of the different groups was found to be not significantly different. This implied that prior to chemotherapeutic treatment, the rats were growing at a similar rate. A significant difference detected in the % gain in weight of the rats during the post-treatment period could therefore be attributed to the treatment. The % gain in weight of the rats post-treatment among the treatment groups, was found to be not significantly different. The chemotherapeutic treatment, thus

did not have any obvious effect on the % gain in weight. This was further supported by the non-significant difference between the pre- and post-treatment % gain in weight of the rats of each individual treatment group. Pyrantel pamoate, however, was the only group which had a significant negative effect ($P < .05$) on the % gain in weight of the rats. The growth rate of the uninfected group was also found to be unaffected by the chemotherapeutic treatment (Appendices 5 and 6).

Both the uninfected and infected animals' appetites were not affected by the treatment, when tested statistically (Appendices 5 and 6).

The mean weight of the rats and the mean weight of rat chow eaten by the uninfected and infected rats are shown in Tables 9 and 10. Figures 7, 8, 9, and 10 trace the weight of the rats and the weight of the rat chow eaten by the infected and uninfected rats over the experimental period.

The % reduction of adult N. brasiliensis in rats effected by the individual doses of M. azedarach, Q. indica, and pyrantel pamoate were 21%, 26%, and 100% respectively (Table 11, Figure 11, and Appendix 7).

Testing with the ANOVA gave the following :

- (i) There was a highly significant ($P < .001$) added component due to treatment effects in the mean square among groups (treatment).
- (ii) The a priori testing supported the above since

$$F_s = \frac{MS \text{ (control versus treatment)}}{MS_{\text{within}}} = 27.44 \quad ***$$

Table 9: Experiment 2 - The daily mean weight of the rats and the mean weight of rat chow eaten by uninfected rats.

Treatment	Mean weight of uninfected rats (g)				Mean weight of rat chow eaten by uninfected rats (g)			
Day of experiment	Control	<u>M.</u> <u>azedarach</u>	<u>Q.</u> <u>indica</u>	Pyrantel pamoate	Control	<u>M.</u> <u>azedarach</u>	<u>Q.</u> <u>indica</u>	Pyrantel pamoate
0	109.3	105.2	103.1	101.1	14.2	12.8	15.6	17.7
1	119.9	116.3	120.2	118.3	17.9	16.3	12.9	19.9
2	129.2	124.2	128.3	127.2	16.2	15.2	17.4	17.6
3	137.4	132.5	134.3	135.1	19.8	17.1	16.4	18.5
4	144.4	137.9	141.5	141.1	-	-	-	-
5	154.5	150.1	150.6	149.8	22.4	19.6	19.2	19.2
6	159.6	153.7	157.0	157.2	22.4	20.7	20.7	21.5
7	169.4	162.1	164.8	163.9	19.9	18.2	18.5	17.6
8	180.0	168.4	171.1	168.6	18.3	17.1	17.8	18.3
9	185.3	173.5	175.0	173.1	20.9	18.5	18.8	18.2

Table 10: Experiment 2 - The daily mean weight of the rats and the mean weight of rat chow eaten by infected rats.

Treatment	Mean weight of infected rats (g)				Mean weight of rat chow eaten by infected rats (g)			
Day post-infection	Control	<u>M.</u> <u>azedarach</u>	<u>Q.</u> <u>indica</u>	Pyrantel pamoate	Control	<u>M.</u> <u>azedarach</u>	<u>Q.</u> <u>indica</u>	Pyrantel pamoate
0	105.5	108.4	105.7	102.1	14.8	15.7	16.6	11.9
1	113.1	115.3	116.7	110.1	15.1	17.2	17.8	15.4
2	122.2	123.7	125.3	118.4	15.9	16.1	18.4	17.5
3	130.3	131.3	135.2	125.1	16.7	17.2	18.5	16.6
4	139.7	139.0	141.4	134.5	-	-	-	-
5	147.0	148.0	150.1	143.5	16.8	20.7	19.2	19.3
6	154.1	157.1	160.8	150.8	19.7	19.9	21.7	21.0
7	161.5	162.5	166.4	158.8	18.1	18.3	19.9	21.1
8	171.4	170.3	175.5	165.5	17.7	18.1	19.1	17.4
9	179.1	176.6	181.1	171.9	20.2	19.8	20.7	20.2

Figure 7: Experiment 2 - The effect of M. azedarach (seed), Q. indica, and pyrantel pamoate on the weight of uninfected rats.

(An arrow indicated the administration of chemotherapeutic treatment on that particular day).

Key:

Control	—●—●—●—
<u>M. azedarach</u>	—▲—▲—▲—
<u>Q. indica</u>	—⊖...⊖...⊖...⊖—
Pyrantel pamoate	—★---★---★---

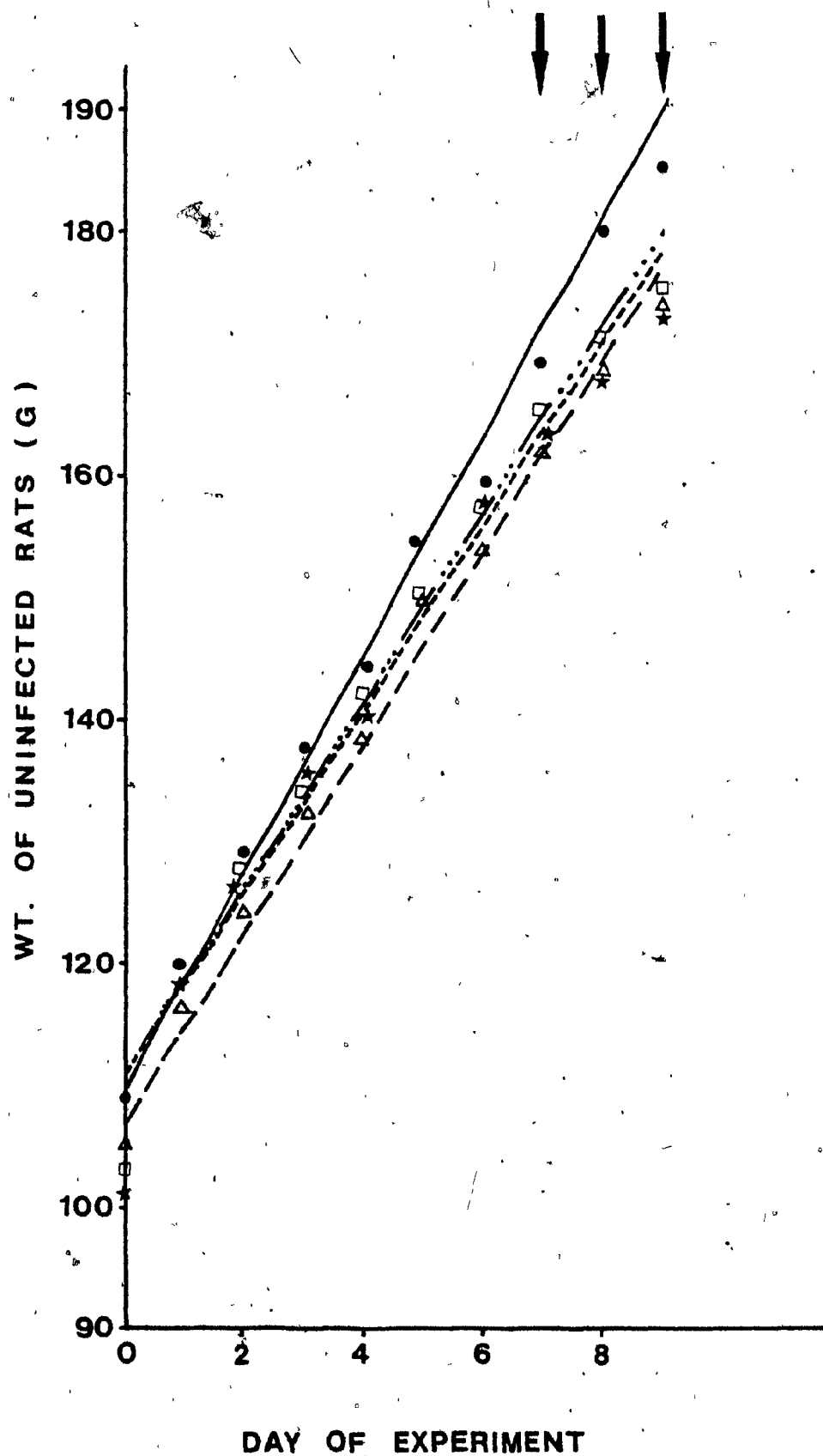


Figure 8: Experiment 2 - The effect of M. azedarach
(seed), Q. indica, and pyrantel pamoate on
the weight of infected rats.

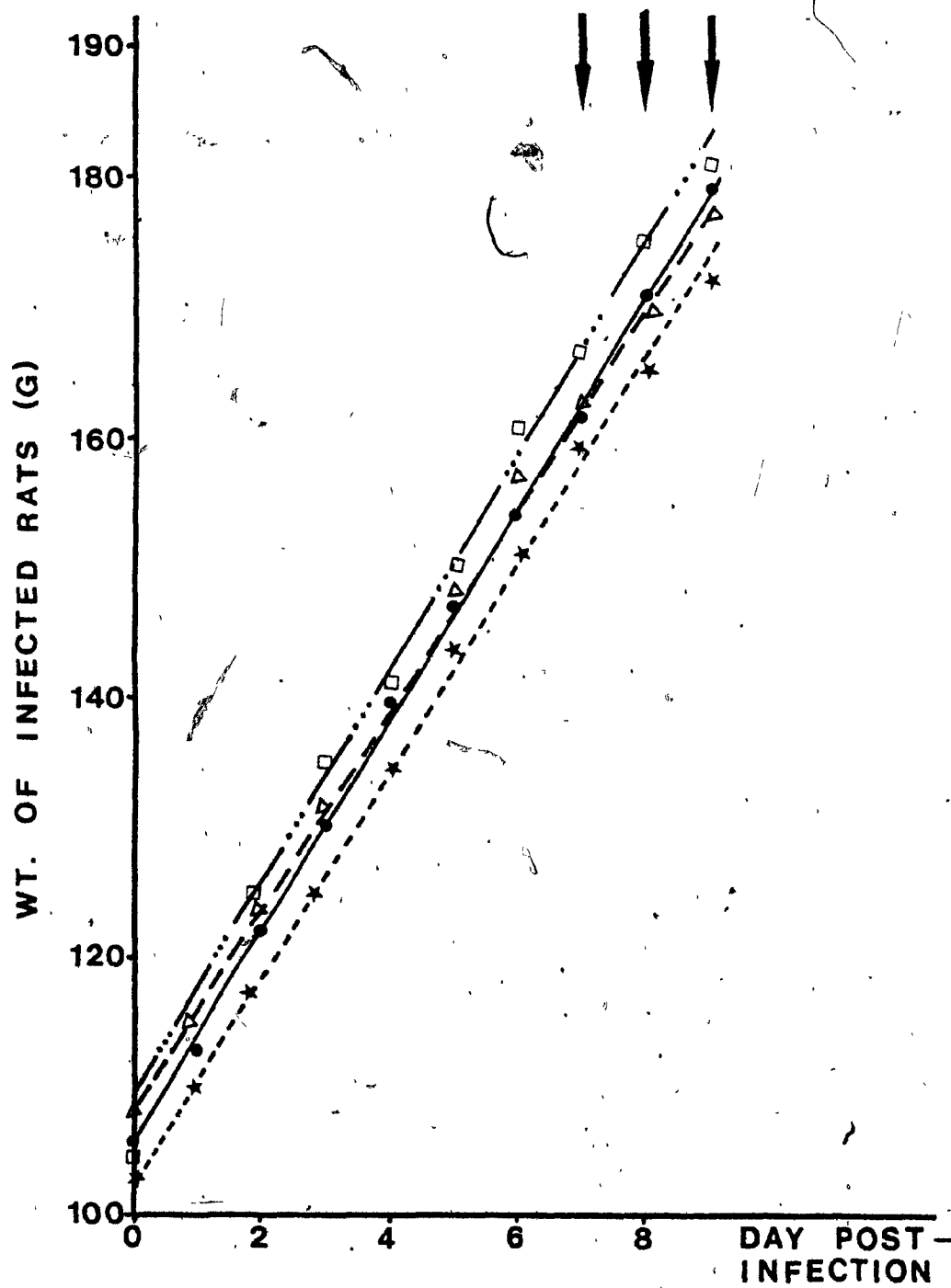


Figure 9: Experiment 2 - The effect of M. azedarach
(seed), Q. indica, and pyrantel pamoate on the
weight of rat chow eaten by uninfected rats.

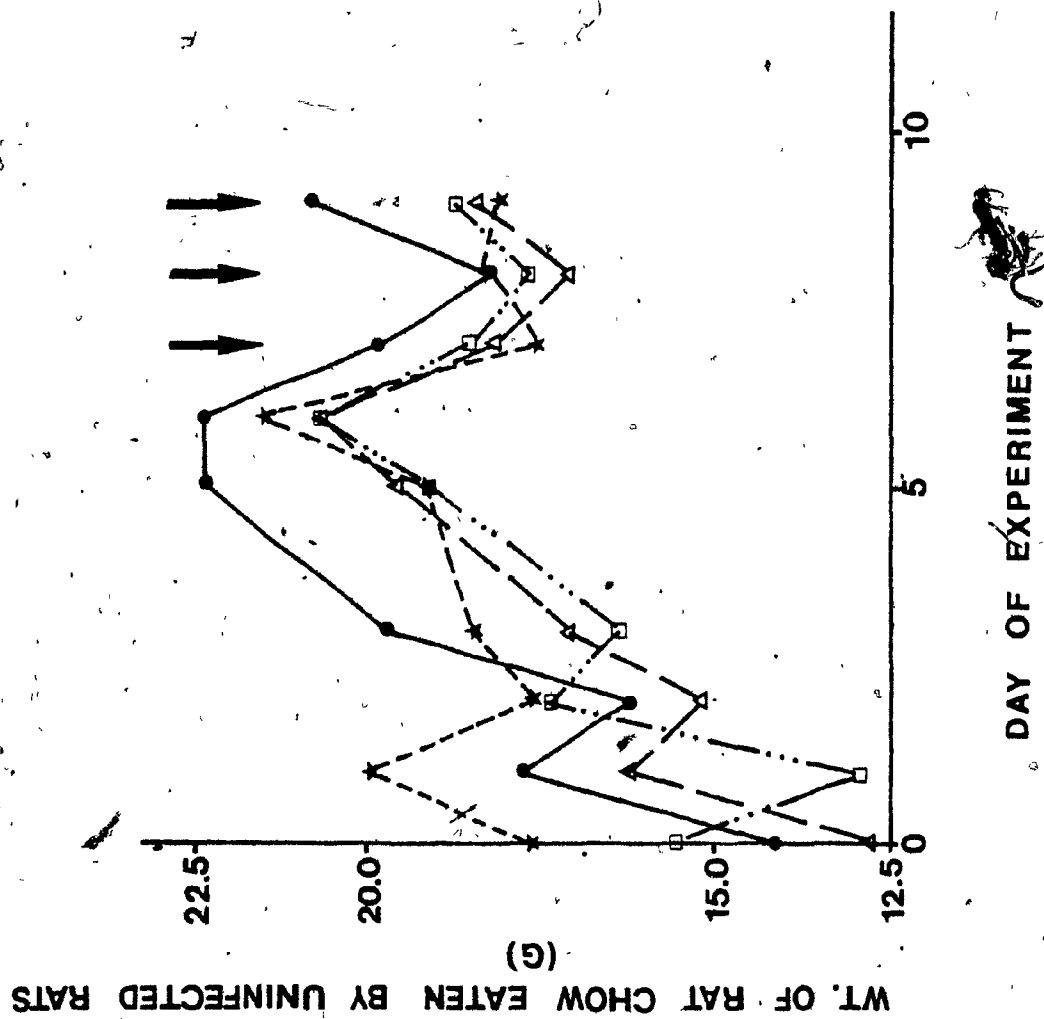


Figure 10: Experiment 2 - The effect of M. azedarach
(seed), Q. indica, and pyrantel pamoate on the
weight of rat chow eaten by infected rats.

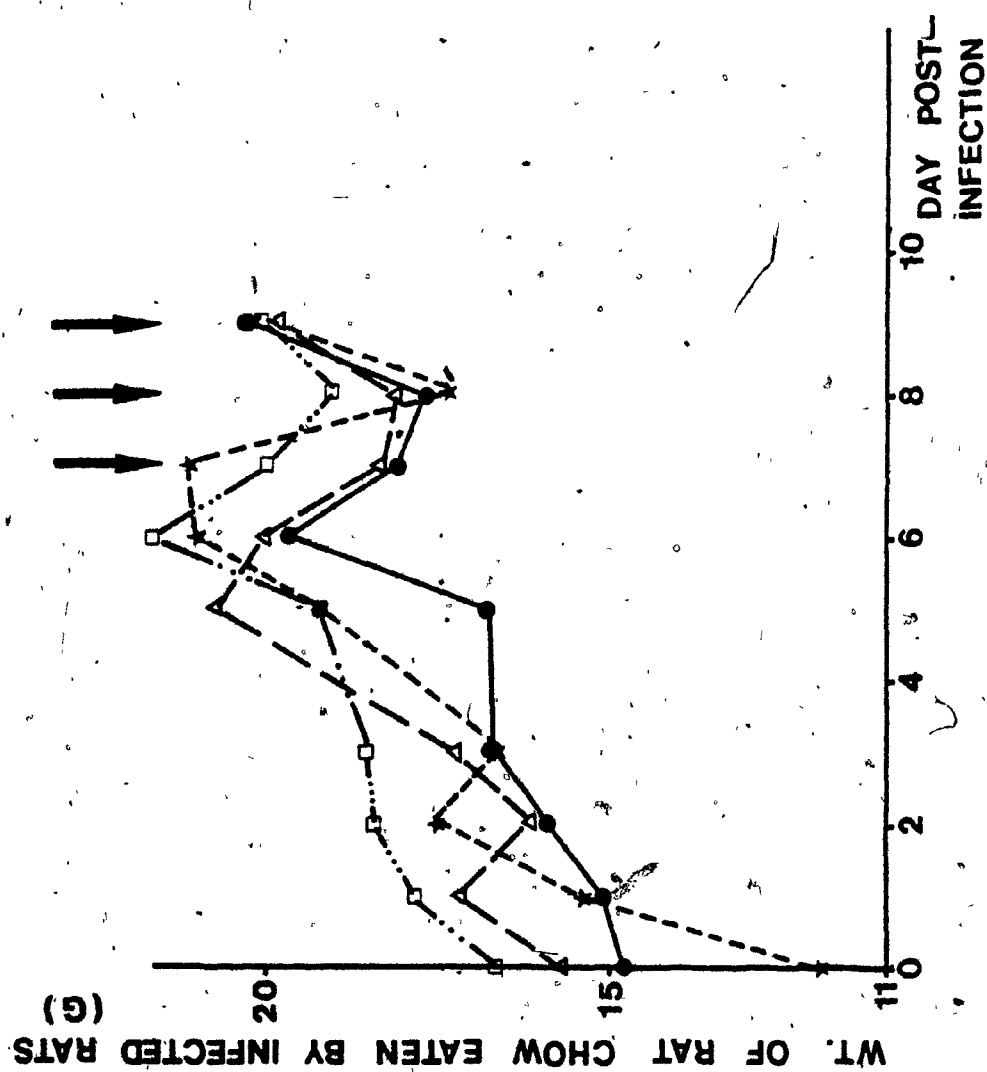
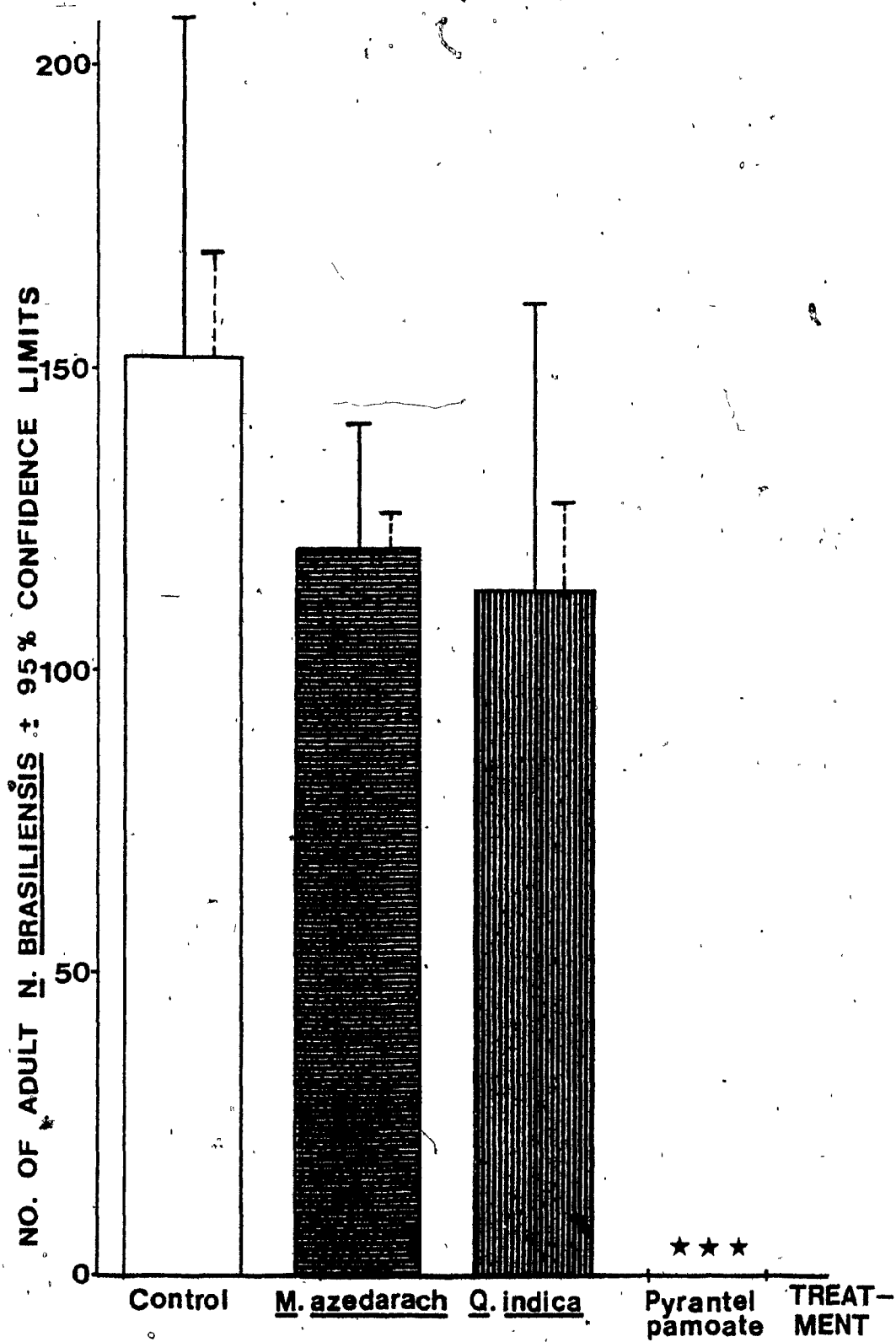


Table 11: Experiment 2 - The mean number of adult N. brasiliensis recovered from the intestines of rats treated with M. azedarach (seed), Q. indica, and pyrantel pamoate ($\pm$ 95% confidence limits and $\pm$ standard error) and % efficacy.

Treatment	Control	<u>M. azedarach</u>	<u>Q. indica</u>	Pyrantel pamoate
Mean no. of adult <u>N. brasiliensis</u> ± 95% confidence limits ± standard error N =	151.3 ± 56.6 151.3 ± 17.8 4	119.5 ± 21.5 119.5 ± 6.8 4	112.5 ± 48.7 112.5 ± 15.3 4	0.0 ± 0.0 0.0 ± 0.0 4
% efficacy	0.0	21.0	25.6	100.0

Figure 11: Experiment 2 - The effect of M. azedarach

(seed), Q. indica, and pyrantel pamoate on the
mean number of adult N. brasiliensis recovered
from the intestines of rats $\pm$ 95% confidence
limits and $\pm$ standard error.



% efficacy:

20.99

25.62

100.00

n :

4

4

4

4

$$F_{.001} (1, 12) = 18.6$$

The chemotherapeutic treatment had a significant effect of the recovery of adult N. brasiliensis since $F_s > F_{.001} (1, 12)$.

This could be due to either pyrantel pamoate and M. azedarach or Q. indica, or both herbs.

$$F_s = \frac{MS (\text{control versus herbs})}{MS_{\text{within}}} = 5.55 *$$

$$F_{.05} (1, 12) = 4.75;$$

$$F_{.01} (1, 12) = 9.33$$

The herbs have a significant effect at $P = .05$ level.

$$F_s = \frac{MS (\text{control versus } \underline{M. azedarach})}{MS_{\text{within}}} = 3.37$$

The significant difference between the control and the herbal treatments was due to the effect of Q. indica and not M. azedarach.

(iii) The results of the a posteriori testing are tabulated in Table 12. The number of adult N. brasiliensis recovered from the rats in the groups treated with Q. indica, M. azedarach, and the control group was not significantly different at the 95% level. Since the a priori test is more sensitive, it is concluded that the anthelmintic effect of Q. indica is significant when compared with the untreated controls and thus warranted further study.

Table 12: A posteriori testing of the results from
Experiment 2, using the SNK procedure.

Treatment	Pyrantel pamoate	<u>Q. indica</u>	<u>M. azedarach</u>	Control
Mean no. of adult <u>N. brasiliensis</u> N. = 4	0.0	112.5	119.5	151.3

3) Experiment 3

(a) Rationale:

Q. indica was used because the previous experiment showed promising results. M. lapidescens was tested since it was used in a mixture in Experiment 1. The infective dose was increased to 500 infective larvae. The heating time for the preparation of the herbs was further reduced so as to minimize the risk of destroying the active ingredients.

(b) Experimental design:

Four treatment groups : control, group treated with Q. indica, M. lapidescens, and pyrantel pamoate were used. They were further subdivided into 2 subgroups : the infected group had 4 replications each while the uninfected group had 3 replications (Table 13).

(c) Materials and methods:

(i) Inoculation of the rats

This followed the method outlined in Experiment 1 except a dose of 500 infective larvae was inoculated into each of the 4 week-old rats used.

(ii) Preparation of the herbs

Fresh preparations of Q. indica and M. lapidescens were made each day before feeding the rats. The herbs were prepared as described in Experiment 2 except the heating time was reduced to about 10 minutes.

Table 13: Experiment 3 - Experimental design.

Treatment	Control	<u>Q. indica</u>	<u>M. lapidescens</u>	Pyrantel pamoate
No. of replications (rats infected with <u>N. brasiliensis</u>)	4	4	4	4
No. of replications (uninfected rats)	3	3	3	3

(iii) Chemotherapeutic trials and evaluation

The rats were weighed daily at 09.00 hour. They were fasted for 6 hours prior to the chemotherapeutic treatment given on days 7, 8, and 9 post-infection.

The rats were sacrificed on day 10 post-infection. The adult parasites were recovered as described in Experiment 1.

(d) Results:

The difference between the pre-treatment and post-treatment growth rates of the rats were found to be not significant in each of the treatment groups ($P = .05$). The chemotherapeutic treatments, thus, did not have an effect on the weight of the rats (Appendices 8 and 9).

The % reductions of adults of N. brasiliensis effected by Q. indica, M. lapidescens, and pyrantel pamoate compared to the controls, were -0.43%, -8.25%, and 99.27% respectively (Table 14, Figure 12, and Appendix 10).

The results of the statistical analyses are listed below.

i) The preliminary ANOVA showed that the treatments were significantly different ($P < .001$).

ii) The a priori testing gave the following results :

$$F_s = \frac{MS \text{ (control versus treatment)}}{MS_{\text{within}}} = 13.22 **$$

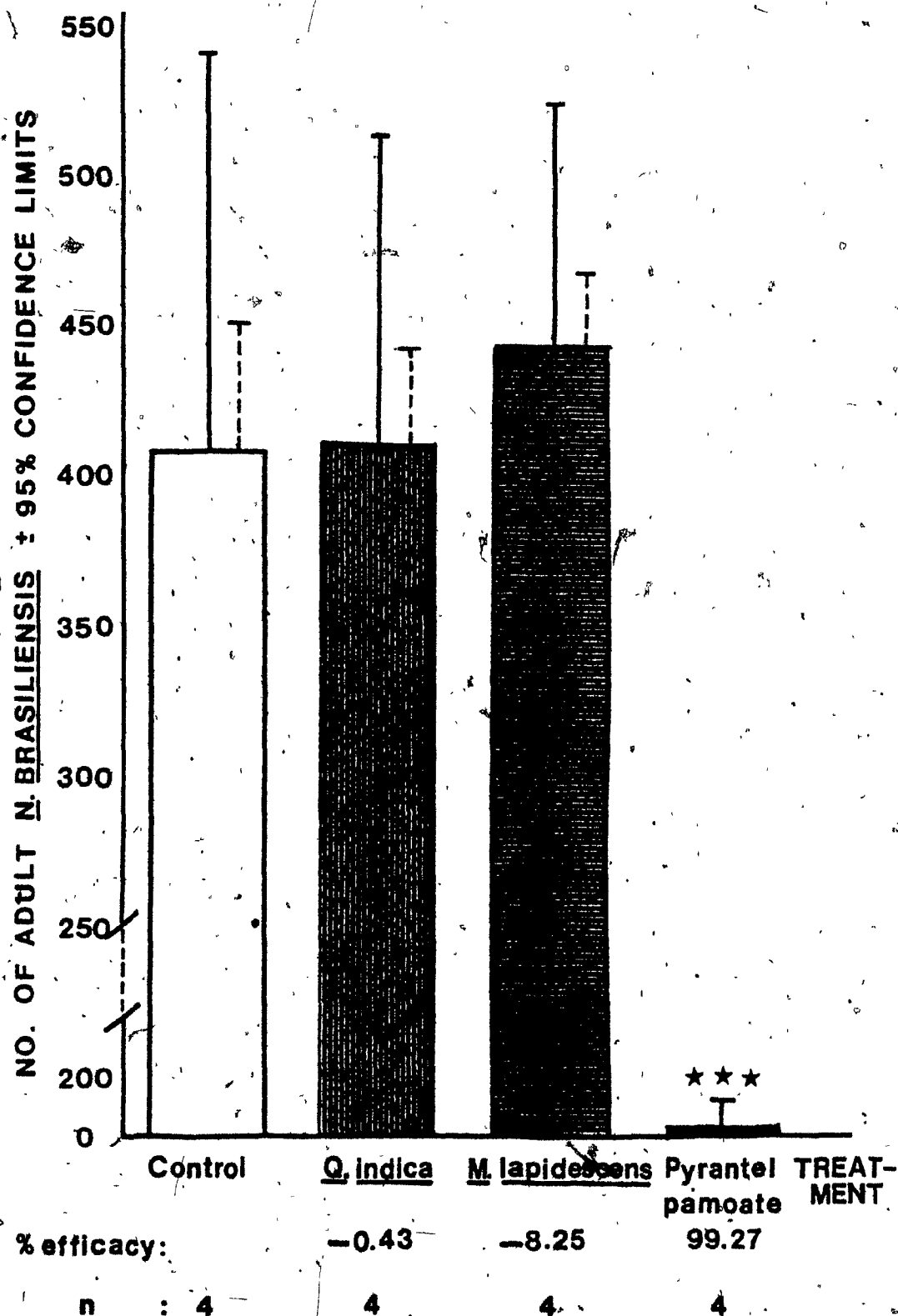
$$F_s = \frac{MS \text{ (control versus herbal treatment)}}{MS_{\text{within}}} = 0.24$$

$$F_{.05} (1, 12) = 4.75;$$

Table 14:.. Experiment 3 - The mean number of adult N. bra-
siliensis recovered from the intestines of rats
treated with M. lapidescens, Q. indica, and
pyrantel pamoate (+ 95% confidence limits and
+ standard error) and % efficacy.

Treatment	Control	<u>Q. indica</u>	<u>M. lapidescens</u>	Pyrantel pamoate
Mean no. of adult <u>N. brasiliensis</u> + 95% confidence limits + standard error N =	 409.0 ± 131.9 409.0 ± 41.5 4	 410.8 ± 102.6 410.8 ± 32.3 4	 442.8 ± 82.1 442.8 ± 25.8 4	 3.0 ± 8.5 3.0 ± 2.7 4
% efficacy	0.0	- 0.4	- 8.3	99.3

Figure 12: Experiment 3 - The effect of M. lapidescens,
Q. indica, and pyrantel pamoate on the mean
number of adult of M. brasiliensis recovered
from the intestines of rats $\pm$ 95% confidence
limits and $\pm$ standard error.



$$F_{.01} (1, 12) = 9.33;$$

$$F_{.001} (1, 12) = 18.60$$

Pyrantel pamoate was the treatment responsible for the significant difference. The chemotherapeutic effects of Q. indica and M. lapidescens were negligible since $F_s = 0.24 \ll F_{.05} (1, 12)$.

iii) The a posteriori testing gave similar conclusions. The reductions of the parasite load by Q. indica and M. lapidescens were not significantly different from that of the control group while pyrantel pamoate significantly reduced the parasite load at the 95% level (Table 15).

M. lapidescens is reputed for its anticestodal properties rather than for its nematodal effects. This could account for the results.

These results from the three experiments did not seem to indicate that heating destroyed any active ingredients since the efficacy of the TCHAs did not increase significantly with the reduction of boiling time.

In Experiments 1, 2, and 3, pyrantel pamoate gave virtually 100% parasite reduction. Unless the modes of action of pyrantel pamoate and the TCHAs were radically different, the low efficacies of the TCHAs were probably not due to the host-parasite system used. It was not necessary to include pyrantel pamoate as the reference drug in subsequent experiments since the results obtained were relatively consistent. The effect of the treatments on the weight and appetite of the rats was not pursued further since the treatment did not seem to elicit any positive

Table 15: A posteriori testing of the results from Experiment 3, using the SNK procedure.

Treatment	Pyrantel pamoate	Control	<u>Q. indica</u>	<u>M. lapidescens</u>
Mean no. of adult <u>N. brasiliensis</u> N = 4	3.0 409.0 410.8 442.8			

response in either of the characteristics. The TCHAs tested thus did not damage the 'health' of the rats, as indicated by the parameters measured.

4) Experiment 4

(a) Rationale:

A. catechu and C. pepo, Q. indica, M. azedarach, and combinations of these were screened. This was designed to study the synergistic and antagonistic effects of the herbs.

(b) Experimental design:

Eight treatment groups : control, A. catechu with C. pepo (a), Q. indica (b), M. azedarach (c), (ab), (bc), (ac), and (abc), were set up. Each treatment group had 3 replications which was the number of animals that can be handled (Table 16).

(c) Materials and methods:

(i) Inoculation of the rats

An inoculation of 1000 infective larvae was used. The method of inoculation was similar to that described in Experiment 1.

(ii) Preparation of the herbs

A. catechu (288 g) and C. pepo (240 g) were pounded and ground in a blender for about 30 s. The efficiency of the Soxhlet Extraction apparatus (Plate 4) was tested as an alternative method of preparation. Water (200 ml) was boiled in a 250 ml round-bottomed flask.

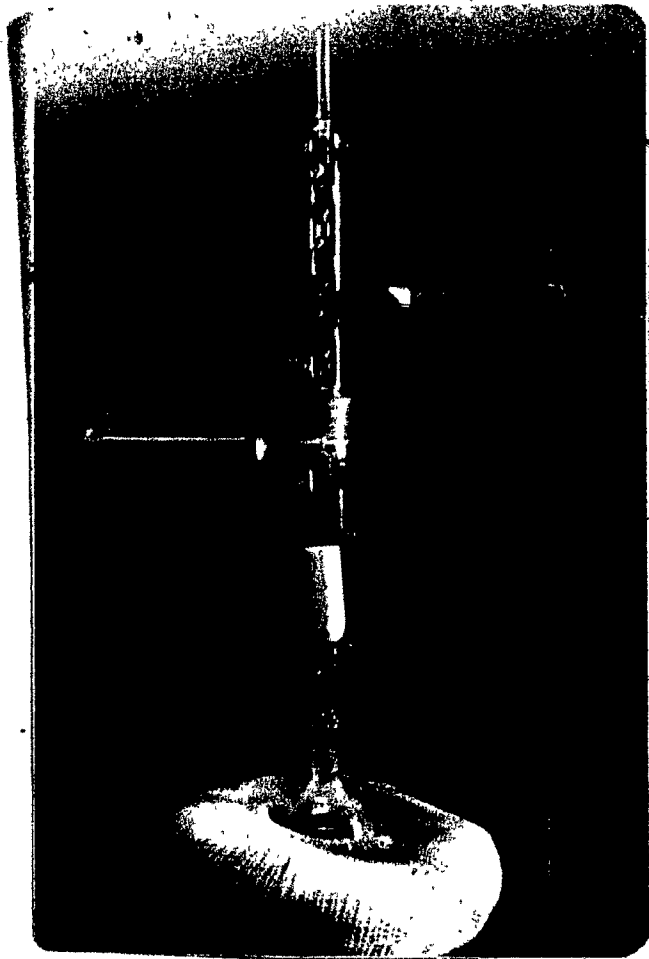
Table 16: Experiment 4 - Experimental design.

Treatment	Control	a	b	c	ab	bc	ac	abc
No. of replications (rats)	3	3	3	3	3	3	3	3

where

- a = A. catechu and C. pepo;
- b = Q. indica;
- c = M. azedarach (root);
- ab = 1:1 mixture of A. catechu and C. pepo, and Q. indica;
- bc = 1:1 mixture of Q. indica and M. azedarach;
- ac = 1:1 mixture of A. catechu and C. pepo, and M. azedarach;
- abc = 1:1:1 mixture of A. catechu and C. pepo, Q. indica, and M. azedarach.

Plate 4: Soxhlet Extraction Apparatus.



The evaporated water condensed and filtered through the thimble (Whatman 30 X 77 mm) filled with ground A. catechu and C. pepo, thoroughly mixed. The thimble was changed when the filtered solution was continuously light-coloured. The solution was concentrated to 50 ml, giving a final concentration of 10.56 g A. catechu and C. pepo/ml. This concentration was twelve times that recommended for humans (Chung and Ko, 1973).

Twenty-seven grams of finely ground M. azedarach (bark) was extracted with 62.5 ml water as described above. The final volume of the solution was 50 ml. The concentration of the decoction was 0.54 g M. azedarach/ml.

Twenty pieces of Q. indica without shells weighed about 10.8 g. The finely ground seeds (27 g) were infused with 65 ml of water, using the Soxhlet Extractor, as previously described. The solution was concentrated to 50 ml, resulting in 0.54 g Q. indica/ml (Prescriptions 9 and 10, Part I).

The solutions were refrigerated in sterilized bottles at 4°C and were used throughout Experiment 4 for convenience.

The combinations were made by mixing equal volumes of the herb infusions prior to feeding the rats daily.

(iii) Chemotherapeutic trials and evaluation

The rats were treated on days 7, 8, and 9 post-infection as described in Experiment 1.

The rats were fasted for 6 hours before they were sacrificed on day 10 post-infection. The adult parasites were recovered as in Experiment 1.

(d) Results:

The % reductions of the parasite load compared to the control of the treatments a, b, c, ab, bc, ac, and abc (Table 17, Figure 13, and Appendix 11) were 0%, 5.98%, -19.49%, -34.01%, -12.25%, -24.45%, and -22.35%, respectively. There was a significant ($P < .05$) added variance component among treatments on the parasite load.

The a priori testing gave the following results :

$$i) F_s = \frac{MS \text{ (control versus treatment)}}{MS_{\text{within}}} = 3.97$$

This is not significant since $F_s < F_{.05}(1, 16) (=4.49)$.

$$ii) F_s = \frac{MS \text{ (single herbs versus combinations)}}{MS_{\text{within}}} = 11.80 **$$

The effect of single herbs was significantly different from that of combinations since $F_{.01}(1, 16) = 8.53$.

The a posteriori comparisons are summarized in Table 18. The treatments b, control, a, bc, c, abc, and ac are not significantly different at the 95% level, while the treatments bc, c, abc, ac, and ab are not significantly different at the 95% level.

Ranking the means, it was noted that antagonistic and synergistic interactions could have occurred. Theoretical values of the interactions and their 95% confidence limits were calculated by averaging the means of the individual components, and the lower and upper limits at the 95% level.

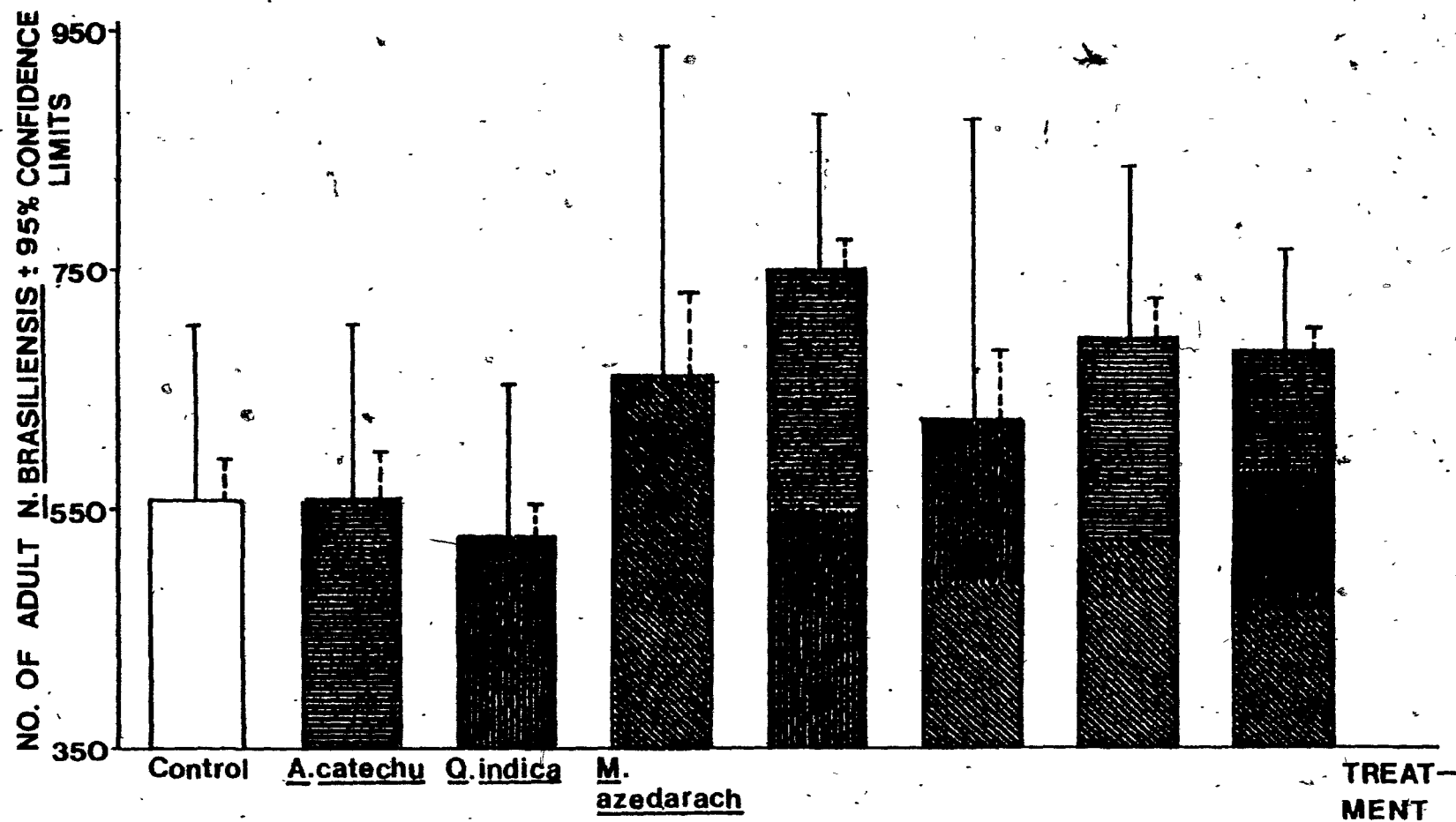
The deduction process is outlined below :-

A. catechu with C. pepo (a) and Q. indica (b), individually resulted

Table 17: Experiment 4 - The mean number of adult N. bra-
siliensis recovered from the intestines of rats
treated with combinations of A. catechu and
C. pepo, M. azedarach (root), and O. indica
(+ 95% confidence limits and + standard error)
and % efficacy.

Treatment	Control	a	b	c	ab	bc	ac	abc
Mean no. of adult <u>N. brasiliensis</u>	557.7	557.7	524.3	666.3	747.3	626.0	694.0	682.3
$\pm$ 95% confidence limits	$\pm$ 143.3	$\pm$ 145.5	$\pm$ 127.8	$\pm$ 272.2	$\pm$ 131.0	$\pm$ 245.8	$\pm$ 136.6	$\pm$ 83.2
$\pm$ standard error	$\pm$ 33.4	$\pm$ 33.8	$\pm$ 29.7	$\pm$ 63.6	$\pm$ 30.5	$\pm$ 57.2	$\pm$ 31.8	$\pm$ 19.3
N =	3	3	3	3	3	3	3	3
% efficacy	0.0	0.0	6.0	- 19.5	- 34.0	- 12.3	- 24.5	- 22.4

Figure 13: Experiment 4 - The effect of different combinations of A. catechu and C. pepo, M. azedarach, and Q. indica on the mean number of adult N. brasiliensis recovered from the intestines of rats $\pm$ 95% confidence limits and $\pm$ standard error.



	<u>C. pepo</u>						
	a	b	c	ab	bc	ac	abc
% efficacy:	0.00	5.98	-19.49	-34.01	-12.25	-24.45	-22.35
n : 3	3	3	3	3	3	3	3

Table 18: A posteriori testing of the results from
Experiment 4, using the SNK procedure.

Treatment	b	Control	a	bc	c	abc	ac	ab
Mean no. of adult <u>N. brasiliensis</u> N = 3	524.3	557.7	557.7	626.0	666.3	682.3	694.0	747.3

in a recovery of 557.7 and 524.3 adult N. brasiliensis. If there were no interacting effects, theoretically, the mean number of N. brasiliensis, when treated with ab, would be $\frac{1}{2} (557.7 + 524.3) = 541.0$, and the 95% confidence intervals would be between $\frac{1}{2} (412.2 + 396.5) = 404.4$, and $\frac{1}{2} (703.2 + 652.1) = 677.6$. Since the mean experimental value for the group treated with ab was 747.3, and it lies within the theoretical limits at the 95% level of 616.3 and 878.3, it could be concluded that there were no interacting effects.

The other combinations were analysed similarly. The interacting effects of b and c, a and c, a and bc, b and ac, and c and ab, were not significant at the 95% level.

Several herbs prescribed in the traditional Chinese herbal prescriptions would seem redundant; various combinations of these herbs were not synergistic. The effect of single herbs would be as efficacious as a mixture of them for single infections.

A. catechu with C. pepo are known for their anticestodal properties. M. azedarach had been and still is recommended for nematode infections, especially against A. lumbricoides by Chinese medical texts (Part I, Section C, pp. 37-55). From the experimental results, the anthelmintic efficacy of M. azedarach, using the laboratory model, seems overrated. Q. indica remained the only candidate among the herbs screened which warranted further study on its anti-nematodal character. The worm burden of treated rats, however, was not significantly different from that of the untreated controls. It was thus postulated that the dose concentration may not have been sufficiently

high.

The Soxhlet Extractor was not efficient in extracting more of the active ingredients, if there were any present, since the % efficacy was not increased.

5) Experiment 5

(a) Rationale:

This experiment examined the effect of dose concentration of Q. indica, the most promising herb, on the parasite load.

(b) Experimental design:

In the previous experiments, a multiple factor of 12 was applied of the human concentration. Since the aim of this experiment was to investigate the effect of the concentration of Q. indica on the parasite load, the following 4 treatment groups : control, group treated with 5X Q. indica, (Appendix 2 - dosage calculations), group treated with 10X Q. indica, and group treated with 20X Q. indica, with 4 replications each, were set up (Table 19). The concentration 5X represented five times that of the dose recommended for humans (Materials and methods 5 (c) (ii)).

(c) Materials and methods:

(i) Inoculation of the rats

An inoculum of 1000 infective larvae was injected into each rat subcutaneously as described in Experiment 1.

Table 19: Experiment 5 - Experimental design.

Treatment	Control	Concentration of <u>Q. indica</u> (g/ml)		
		0.225	0.450	0.900
No. of replications (rats)	4	4	4	4

(ii) Preparation of the herbs

Q. indica was shelled and 216 g were pounded. This was boiled in a covered clay pot with 300 ml water, while wrapped in cotton gauze. The evaporated water vapour condensed and filtered through the cotton gauze-wrapped Q. indica. This works on a similar principle as the Soxhlet Extractor, except it is faster and allows larger particles to filter through since it has a larger surface area and the gauze has larger pores than the Whatman filter size 30 X 77 mm. Boiling continued for 2 hours, until 240 ml of solution remained. The final concentration was 0.9 g Q. indica/ml (equivalent to 20X human dose). The solution was diluted 1:1 volume to give a concentration of 0.45 g Q. indica/ml (equivalent to 10X human dose). This diluted solution was further diluted 1:1 volume, resulting in a concentration of 0.225 g Q. indica/ml. The dilutions were made daily prior to the chemotherapeutic treatment. The concentrated solution was refrigerated at 4°C.

(iii) Chemotherapeutic trials and evaluation

The amount of Q. indica extract given to the rats was calculated on a weight to weight basis on the assumption that a 60 kg human was given 240 ml of 0.045 g Q. indica extract/ml.

The rats were treated on days 7, 8, and 9 post-infection and sacrificed on day 10.

The recovery of adult N. brasiliensis was described in Experiment 1.

(d) Results:

The % reductions of adult N. brasiliensis, as compared to the

controls, of 0.225 g Q. indica/ml, 0.450 g Q. indica/ml, and 0.900 g Q. indica/ml, were 16.59%, 19.70%, and 29.50% (Table 20, Figure 14, and Appendix 12). A sigmoid was plotted of the number of adult N. brasiliensis (Y - axis) against the concentration of Q. indica (X - axis).

The null hypothesis was accepted since $F_s < F_{.05}(3, 12)$.

The a priori testing, however, indicated that the chemotherapeutic treatments significantly ($P < .05$) reduced the parasite load. The effect of the different concentrations was not significantly different. The a posteriori (SNK) procedure gave similar results (Table 21).

The a priori test is a sensitive test. Although a nonsignificant overall analysis of variance was obtained, the a priori and a posteriori comparisons, were significant. The sample size may not have been large enough to detect the significance.

6) Experiment 6

(a) Rationale:

This experiment was performed to increase the number of replications.

(b) Experimental design:

It was similar to that described for Experiment 5 (Table 19).

(c) Materials and methods:

They were as described in Experiment 5.

Table 20: Experiment 5 - The mean number of adult N. brasiliensis recovered from the intestines of rats treated with different concentrations of Q. indica (+ 95% confidence limits and + standard error) and % efficacy.

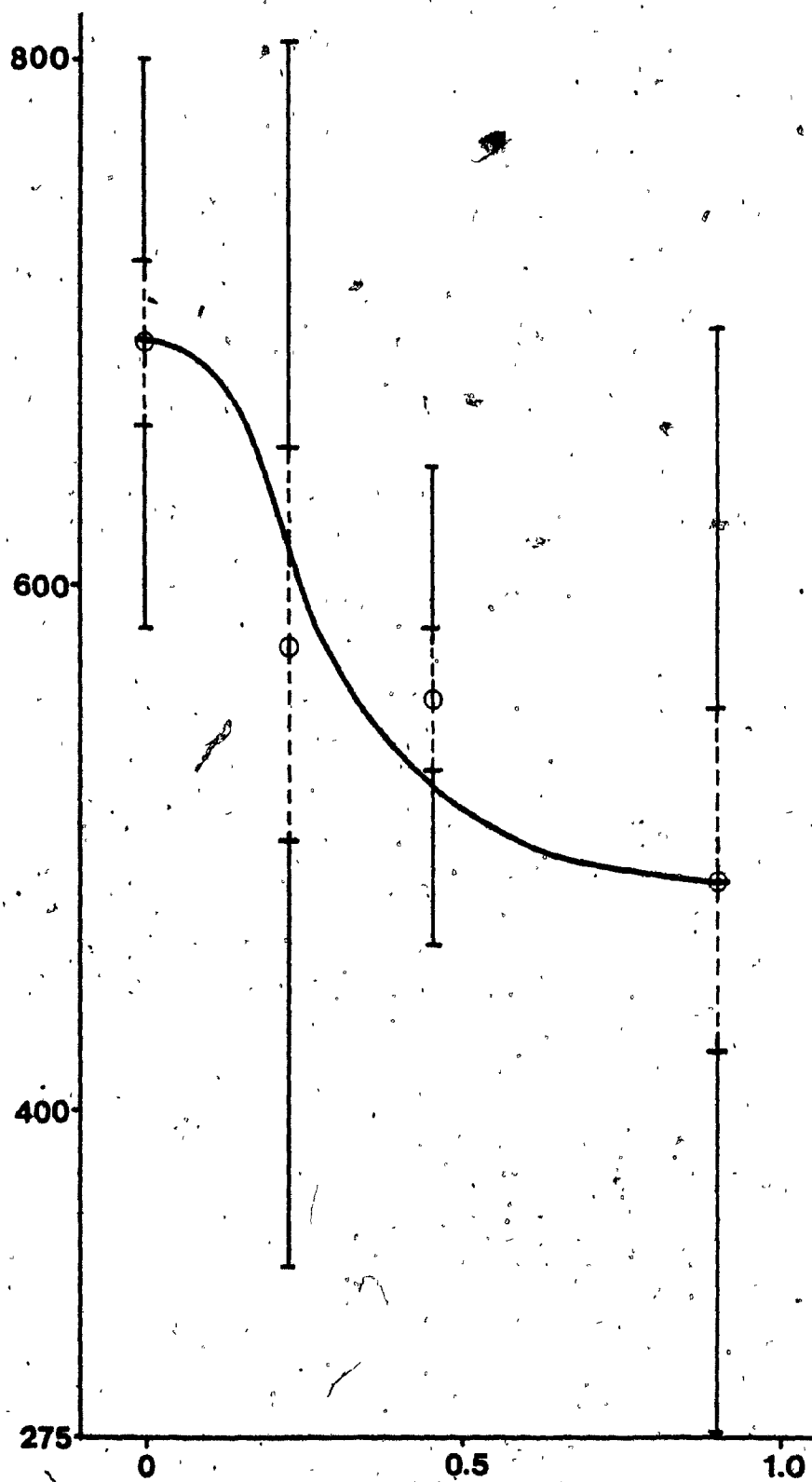
(The sigmoid curve was fitted by eye).

Treatment	Control	Concentration of <u>Q. indica</u> (g/ml)		
		0.225	0.450	0.900
Mean no. of adult <u>N. brasiliensis</u>				
+ 95% confidence limits	691.5 ± 107.8	576.8 ± 236.1	555.3 ± 88.8	487.5 ± 212.2
+ standard error	691.5 ± 31.2	576.8 ± 74.2	555.3 ± 27.9	487.5 ± 66.7
N =	4	4	4	4
% efficacy	0.0	16.6	19.7	29.5

Figure 14: Experiment 5 - The effect of different concentrations of Q. indica on the mean number of adult N. brasiliensis recovered from the intestines of rats $\pm$ 95% confidence limits and $\pm$ standard error.

(The sigmoid curve was fitted by eye).

NO. OF ADULT N. BRASILIENSIS $\pm$ 95% CONFIDENCE LIMITS



CONCENTRATION OF Q. INDICA (G/ML)

Table 21: A-posteriori testing of the results from
Experiment 5, using the SNK procedure.

Treatment	Concentration of <u>Q. indica</u> (g/ml)			Control
	0.900	0.450	0.225	
Mean no. of adult <u>N. brasiliensis</u> N = 4	487.5	555.3	576.8	691.5

(d) Results:

The % reductions of parasite load, as compared to that of the control, by 0.225 g Q. indica/ml, 0.500 g Q. indica/ml, 0.900 g Q. indica/ml were 8.48%, 29.25%, and 31.51% (Table 22, Figure 15, Appendix 13).

The preliminary ANOVA was not significant. The a priori and a postiori testings agreed with this.

Before the results from Experiment 5 and 6 could be pooled, they had to be tested for homogeneity. They were found to be not significantly different ($P < .05$).

The analyses of the pooled results are summarized below :-

(i) The % reductions of adult N. brasiliensis, as compared to the controls, by 0.225 g Q. indica/ml, 0.450 g Q. indica/ml, and 0.900 g Q. indica/ml, were 12.5%, 24.49%, and 30.51% (Table 23, Figure 16, Appendix 14).

(ii) A sigmoid curve seemed to describe the relationship between the number adult N. brasiliensis recovered (Y - axis) and the concentration of Q. indica (X - axis).

(iii) The preliminary ANOVA showed that the differences attributed to the treatment were significant at $P < .01$.

(iv) The a priori testing showed that

$$F_s = \frac{MS \text{ (control versus treatment)}}{MS_{\text{within}}} = 9.93^{**}$$

$$F_{.05} (1, 28) = 4.20$$

$$F_{.01} (1, 28) = 7.64;$$

Table 22: Experiment 6 - The mean number of adult N. bra-
siliensis recovered from the intestines of rats
treated with different concentrations of Q. in-
dica ($\pm$ 95% confidence limits and $\pm$ standard
error) and % efficacy.

Treatment	Control	Concentration of <u>Q. indica</u> (g/ml)		
		0.225	0.450	0.900
Mean no. of adult <u>N. brasiliensis</u> + 95% confidence limits + standard error N =	695.8 ± 215.3 695.8 ± 67.7 4	636.8 ± 170.9 636.8 ± 53.7 4	492.3 ± 267.7 492.3 ± 84.2 4	476.5 ± 258.7 476.5 ± 81.3 4
% efficacy	0.0	8.5	29.3	31.5

Figure 15: Experiment 6 - The effect of different concentrations of Q. indica on the mean number of adult N. brasiliensis recovered from the intestines of rats $\pm$ 95% confidence limits and $\pm$ standard error.
(The sigmoid curve was fitted by eye).

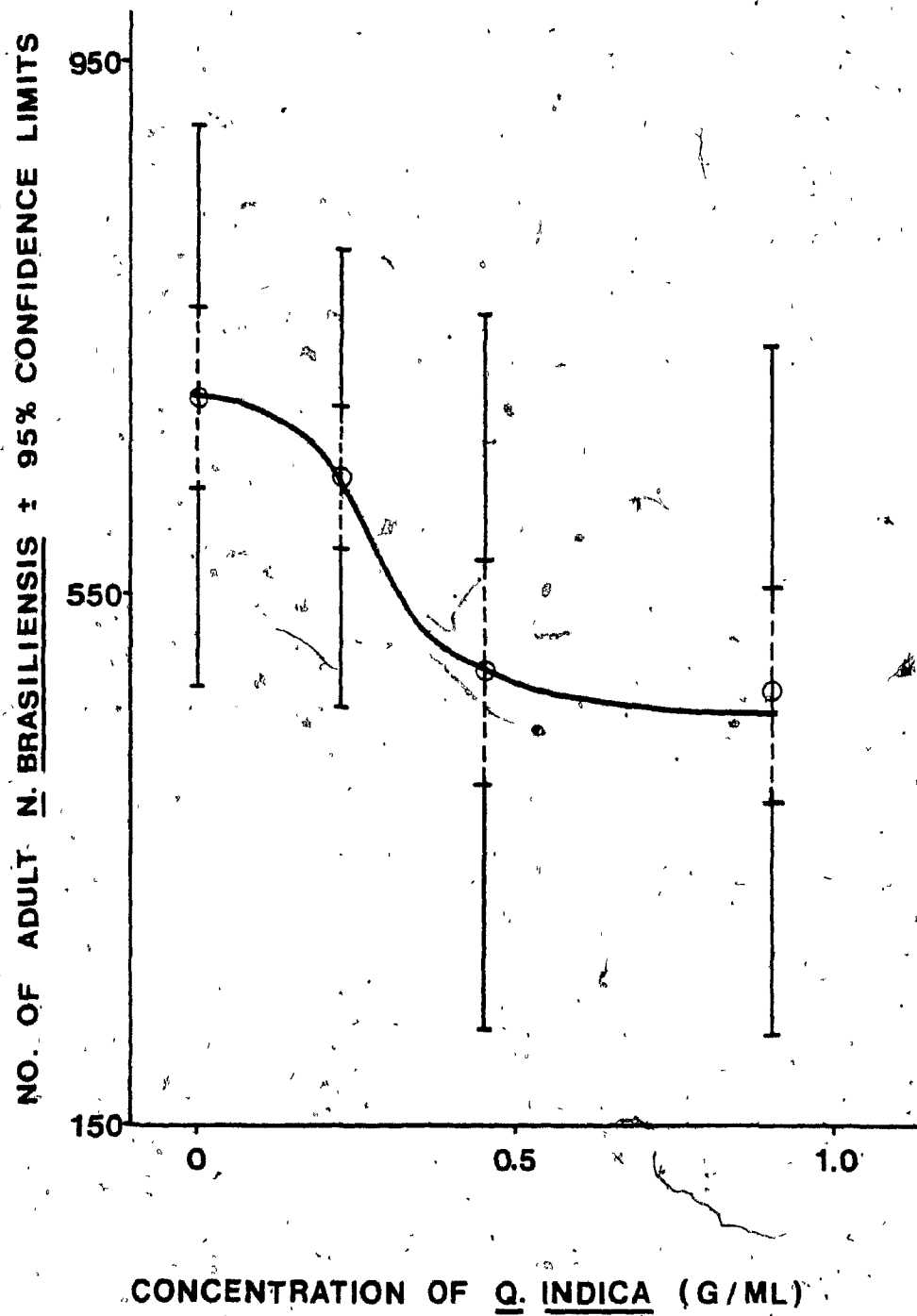
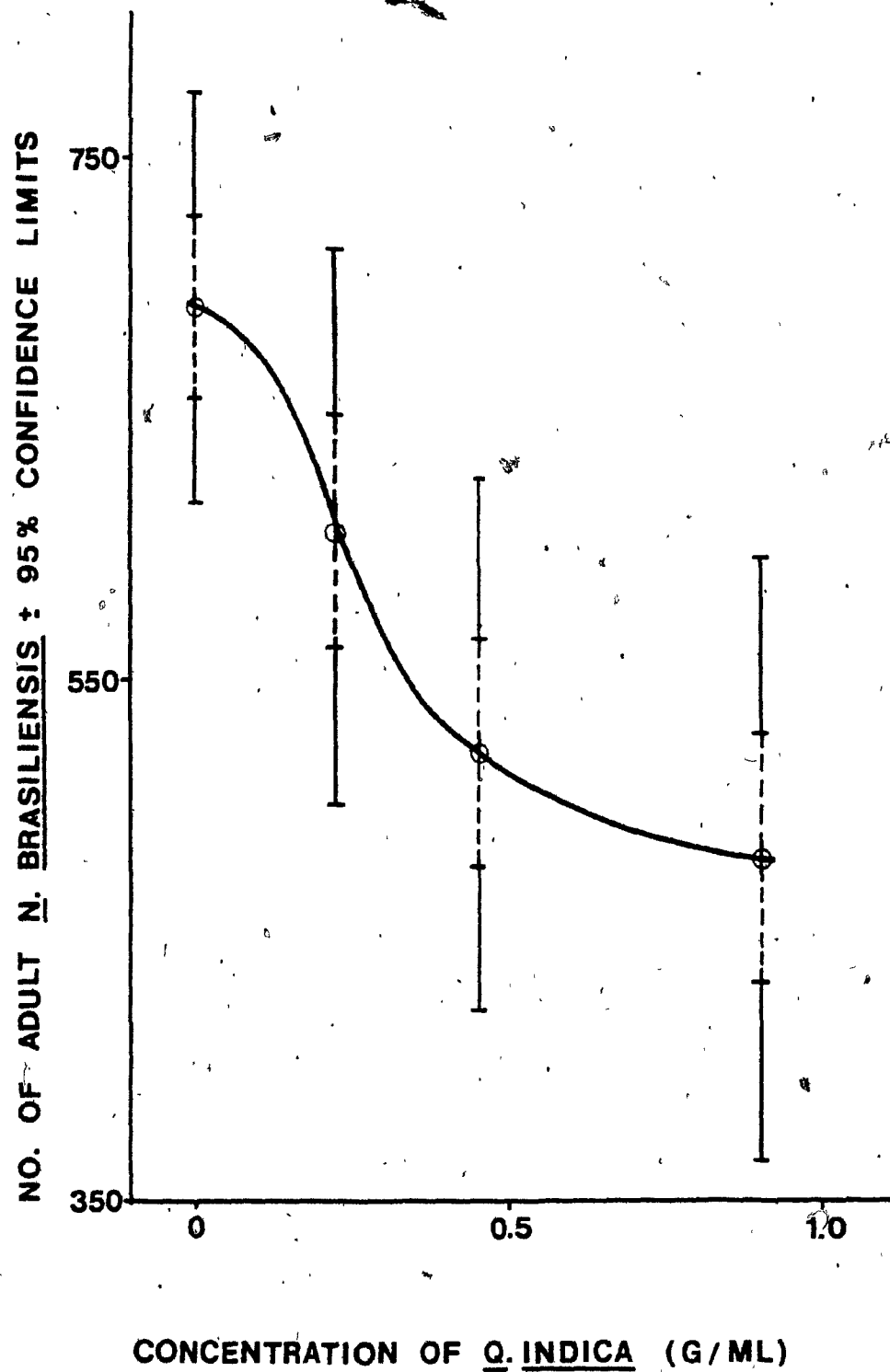


Table 23: The combined results of Experiments of 5 and 6 -
The mean number of adult N. brasiliensis re-
covered from the intestines of rats treated with
different concentrations of Q. indica ($\pm$ 95%
confidence limits and $\pm$ standard error) and
% efficacy.

Treatment	Control	Concentration of <u>Q. indica</u> (g/ml)		
		0.225	0.450	0.900
Mean no. of adult <u>N. brasiliensis</u>				
+ 95% confidence limits	693.6 ± 82.7	606.8 ± 103.6	523.8 ± 100.9	482.0 ± 115.0
+ standard error	693.6 ± 35.1	606.8 ± 43.8	523.8 ± 42.8	482.0 ± 48.7
N =	8	8	8	8
% efficacy	0.0	12.5	24.5	30.5

Figure 16: The combined results of Experiments 5 and 6 -
The effect of different concentrations of
Q. indica on the mean number of adult N. bra-
siliensis recovered from the intestines of rats
+ 95% confidence limits and + standard error.
(The sigmoid curve was fitted by eye).



$$F_{.001} (1, 28) = 13.50$$

This comparison is significant ($.01 > P > .001$), indicating that Q. indica significantly reduced the parasite load.

(v) The results of the a posteriori testing are tabulated in Table 24. The effects of the different concentrations were significantly different from that of the control but were not significantly different among themselves. The concentration 0.225 g Q. indica/ml was not significantly different from that of the control. The testing was at the 95% level.

The conclusions of the preliminary ANOVA, a priori, and a posteori testings were similar. It showed that the effect of 0.450 g Q. indica/ml and higher concentrations was significantly different from that of the control and thus the anthelmintic efficacy was significant. The absolute values, as measured by the % reduction of adult N. brasiliensis, was low, i.e. only 30.51% for 0.900 g Q. indica/ml. This value was much lower than the 100% reduction obtained with pyrantel pamoate. Besides, the concentration of pyrantel pamoate used was twelve times that of the human dose, as compared to the twenty times used with Q. indica (0.900 g Q. indica/ml is equivalent to 20X human dose).

The experiments on N. brasiliensis indicated that only Q. indica reduced parasites. Its efficacy, however, was not as high nor as consistent, as that obtained with pyrantel pamoate. Q. indica did not affect the 'health' of the host adversely. The possible explanations will be further expounded in the Discussion and the General Discussion.

Table 24: A posteriori testing of the combined results
of Experiments 5 and 6, using the SNK procedure.

Treatment	Concentration of <u>Q. indica</u> (μ g/ml)			Control
	0.900	0.450	0.225	
Mean no. of adult <u>N. brasiliensis</u> N = 8	482.0	523.8	606.8	693.6

- B) The evaluation of the chemotherapeutic efficacy of *A. catechu* and *C. pepo*, *U. macrocarpa*, niclosamide, and arecoline hydrobromide against adult *H. diminuta* in rats.

General materials and methods

Tribolium confusum infected with cysticercoids, and gravid proglottids of *H. diminuta* were kindly supplied by Dr. D. Mettrick, of University of Toronto, in December, 1977.

The beetles were kept in plastic petri-dishes. Filter papers, occasionally moistened, layered the enriched flour (Five Roses Brand) on which the beetles fed. T. confusum was preferred to Tenebrio molitor because the former was easier to maintain and its life-span was longer.

The beetles were fasted for 6 hours prior to feeding them with gravid proglottids of *H. diminuta* obtained from the intestines of male Sprague-Dawley albino rats which were dissected. For maintenance, proglottids were left in the culture. However, in the experiments, the uneaten gravid proglottids were removed after 12 hours. This ensured that the infection of the beetles was synchronised, minimizing variation in the cysticercoids. Mature, 17 day-old cysticercoids were used throughout. The cysticercoids were recovered by dissecting the beetles under 0.85% saline. Only undamaged cysticercoids were used.

The rats were lightly etherized. The cysticercoids were fed per os with rounded-tip pasteur pipettes. The pipette was immediately examined for cysticercoids which adhered to the inner wall. This was repeated until all the cysticercoids were in the rat.

Pilot studies showed eggs with hexacanth larvae in the faeces of all the rats by day 18 post-infection. Chemotherapeutic trials were carried out after day 18 post-infection.

After chemotherapeutic treatment, any H. diminuta that survived passed gravid proglottids again about day 14 post-treatment. The rats were sacrificed after at least 14 days post-treatment, to ensure that the scolices and proglottids could be detected.

1) Experiment 7

(a) Rationale:

This experiment tested the efficacies of A. catechu with C. pepo, and U. macrocarpa against H. diminuta, and comparing them with that of niclosamide.

(b) Experimental design:

Four treatment groups: control, group treated with A. catechu and C. pepo, group treated with U. macrocarpa, and group treated with niclosamide, were designed. Each treatment group, originally, had 6 replications. One rat from the control group and the group treated with niclosamide, each did not take the infection. The experimental format which resulted is shown in Table 25.

(c) Materials and methods:

(i) Infection of rats

Cysticercoids (17 day-old) were dissected from T. confusum and fed to 6 week-old male Sprague-Dawley albino rats, as described in

Table 25: Experiment 7 - Experimental design.

Treatment	Control	<u>A. catechu</u> and <u>C. pepo</u>	<u>U. macrocarpa</u>	Niclosamide
No. of replications (rats)	5	6	6	5

General materials and methods. The infective dose was 5 cysticercoids per rat.

(ii) Preparation of the herbs

A. catechu and C. pepo were pounded separately. A. catechu (288 g) and C. pepo (240 g) were well-mixed and wrapped in cotton-gauze. A volume of 200 ml water was initially boiled to make an infusion of A. catechu and C. pepo. An additional 100 ml was added. After 3 hours, a 50 ml infusion of A. catechu and C. pepo was obtained. The concentration of A. catechu was 5.76 g/ml, C. pepo was 4.80g/ml, and the combined concentration was 10.56 g/ml. This was refrigerated at 4°C.

Prescriptions 2 and 6 (Part I) recommended concentrations of 0.0108 g U. macrocarpa/ml and 0.006 g U. macrocarpa/ml, respectively. In many traditional Chinese anthelmintic prescriptions, 10.8 g of herb in 240 ml extract solution is recommended. This would result in a concentration of 0.045 g/ml. Since this concentration was higher than the other two afore-mentioned, this was taken to be the human dose. The rat has a higher metabolic rate. The dose was, therefore multiplied by 12. Pounded U. macrocarpa (129.6 g) was wrapped in cotton-gauze. A volume of 300 ml water was boiled initially, and an additional 450 ml was added later. A 240 ml infusion of U. macrocarpa was obtained in 2 hours. The final concentration of U. macrocarpa was 0.54 g/ml. The infusion was refrigerated at 4°C.

(iii) Chemotherapeutic trials

The chemotherapeutic treatments were given on days 35², 36, and 37 post-infection. The herbs were given on a weight-to-weight

ratio as calculated before (as in Experiment 1). The niclosamide used was kindly donated by Boehringer Ingelheim (Canada) Ltd. (Micronized niclosamide - Lot No. 7831 C, made in Germany). The dose given was 500 mg/(kg wt) (Product Monograph of Niclosamide, published by Boehringer Ingelheim (Canada) Ltd., 1974).

(iv) Evaluation of the chemotherapeutic treatments.

The rats were fasted for 6 hours prior to sacrifice. They were sacrificed on day 76 post-infection. The rats were dissected and the small intestines were removed. Warm saline in a 30 ml glass syringe was used to flush out the contents of the intestines. The small intestines were cut longitudinally and checked, using a dissecting microscope, for any scolices which might be attached to the villi. The number of scolices recovered was noted. This was cross-checked with the number of intact long segments of proglottids. The cestodes were dried in glass petri-dishes lined with pre-weighed aluminium foil. They were dried at 45°C. The constant dry weight was recorded.

(d) Results:

All the rats treated with niclosamide passed out long strands of proglottids about 15 - 30 minutes post-treatment. This was not observed in the other treatment groups.

The % reduction of adult cestodes was calculated by the following formula:

$$\frac{\bar{C}_n - \bar{T}_n}{\bar{C}_n} \times 100$$

where $\bar{C}_n$ = Mean number of adult H. diminuta recovered from the control group and

$\bar{T}_n$ = Mean number of adult H. diminuta recovered from the treatment group.

They were -75.93%, -11.11%, and 100% for the groups treated with A. catechu with C. pepo, U. macrocarpa, and niclosamide, respectively (Table 26, Figure 17, Appendix 15). The preliminary ANOVA was highly significant ($P < .001$). The a priori testing indicated that the control was not significantly different from the herbal treatment. The chemotherapeutic treatments, however, were found to be significantly different. The a posteriori testing is summarized in Table 27.

Table 26, Figure 18, and Appendix 16 showed the results obtained for the dry weight of H. diminuta. The % efficacy was calculated using the following formula :

$$\frac{\bar{C}_{d.w.} - \bar{T}_{d.w.}}{\bar{C}_{d.w.}} \times 100$$

where $\bar{C}_{d.w.}$ = Mean dry weight of adult H. diminuta recovered from the control group and
 $\bar{T}_{d.w.}$ = Mean dry weight of adult H. diminuta recovered from the treatment group.

The results obtained for the groups treated with A. catechu and C. pepo, U. macrocarpa, and niclosamide were -33.71%, -6.82%, and 100%

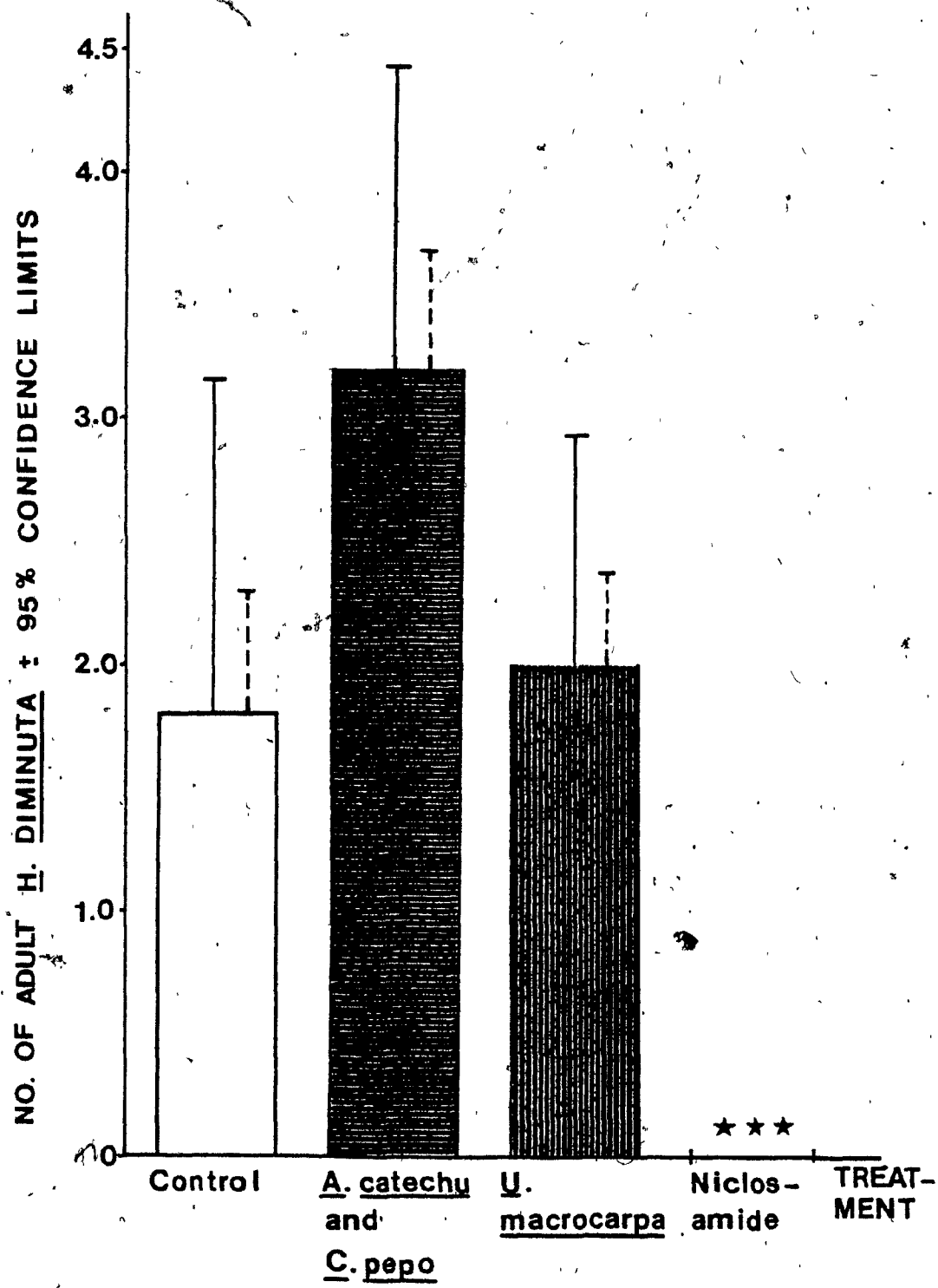
The preliminary ANOVA was highly significant ($P < .001$).

The a priori tests indicated that the control group was not

Table 26: Experiment 7 - The mean number and dry weight
of adult H. diminuta recovered from the intes-
tines of rats treated with A. catechu and
C. pepo, U. macrocarpa, and niclosamide
($\pm$ 95% confidence limits and $\pm$ standard error)
and % efficacy.

Treatment	Control	<u>A. catechu</u> and <u>C. pepo</u>	<u>U. macrocarpa</u>	Niclosamide
Mean no. of adult <u>H. diminuta</u>				
+ 95% confidence limits	1.8 $\pm$ 1.4	3.2 $\pm$ 1.2	2.0 $\pm$ 0.9	0.0 $\pm$ 0.0
+ standard error	1.8 $\pm$ 0.5	3.2 $\pm$ 0.5	2.0 $\pm$ 0.4	0.0 $\pm$ 0.0
N =	5	6	6	5
% efficacy	0.0	- 75.9	- 11.1	100.0
Mean dry weight of adult <u>H. diminuta</u>				
+ 95% confidence limits	0.26 $\pm$ 0.16	0.35 $\pm$ 0.13	0.28 $\pm$ 0.12	0.00 $\pm$ 0.00
+ standard error	0.26 $\pm$ 0.06	0.35 $\pm$ 0.05	0.28 $\pm$ 0.05	0.00 $\pm$ 0.00
N =	5	6	6	5
% efficacy	0.0	- 33.7	- 6.8	0.0

Figure 17: Experiment 7 - The effect of A. catechu and
C. pepo, U. macrocarpa, and niclosamide on
the mean number of adult H. diminuta recovered
from the intestines of rats $\pm$ 95% confidence
limits and $\pm$ standard error.



% efficacy:

- 75.93

- 11.11

100.00

n :

5

6

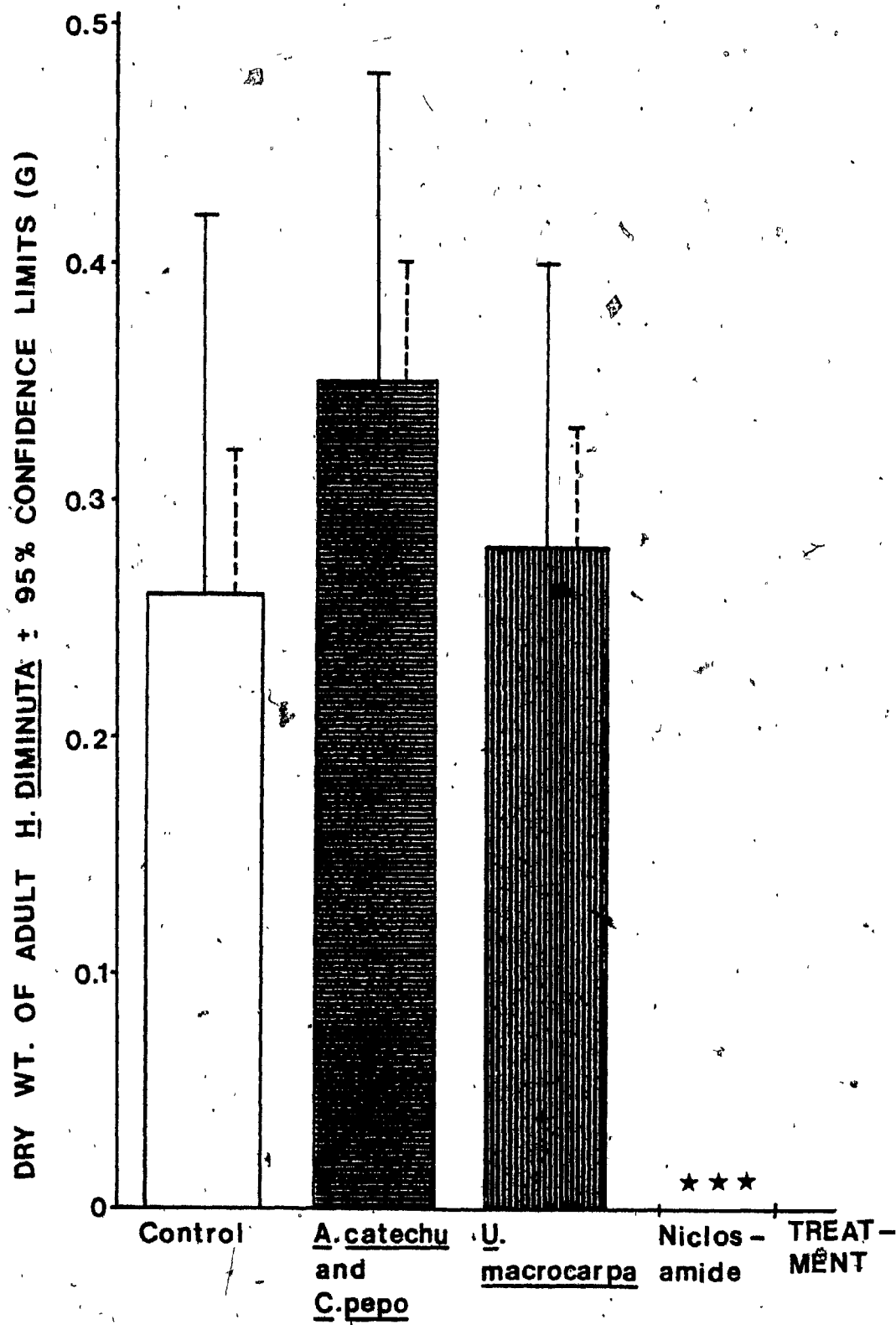
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Table 27: Experiment 7 - A posteriori testing of the mean
number of adult H. diminuta recovered from the
intestines of rats, using the SNK procedure.

Treatment	Niclosamide	Control	<u>U. macrocarpa</u>	<u>A. catechu</u> and <u>C. pepo</u>
Mean no. of adult <u>H. diminuta</u>	0.0	1.8	2.0	3.2
N =	5	5	6	6

Figure 18: Experiment 7 - The effect of A. catechu and
C. pepo, U. macrocarpa, and niclosamide on the
dry weight of adult H. diminuta recovered from
the intestines of rats $\pm$ 95% confidence limits
and $\pm$ standard error.



% efficacy:	-33.71	-6.82	100.00
n :	5	6	5

significantly different from the treatment groups. However,

$$F_s = \frac{MS \text{ (among treatments)}}{MS_{\text{within}}} = 16.21^{**}$$

when $F_{.001}(2, 22) = 9.61$, which implied that the treatment effects were significantly different. The first conclusion of the a priori testing could be explained by the negative % reduction of adult H. diminuta in groups treated with U. macrocarpa, and A. catechu and C. pepo.

The a posteriori testing is summarized in Table 28. The herbal treatments were found to be not significantly different from the control at the 95% level.

The herbal treatments were ineffective and were not significantly different from the control. Niclosamide, in comparison, gave a 100% cure rate. This indicated that the host-parasite system is suitable for the testing of anticestodals, especially against intestinal cestodes, which are ecologically and physiologically similar to Hymenolepididae. It was not necessary in the subsequent experiments to include niclosamide as a treatment group.

2) Experiment 8

(a) Rationale:

The combined effect of A. catechu with C. pepo, and U. macrocarpa was examined in this experiment since they were frequently mentioned in traditional Chinese herbal anthelmintic prescriptions (Prescriptions 10, 12, 13, and 14).

Table 28: Experiment 7 - A posteriori testing of the
mean dry weight of adult H. diminuta recovered
from the intestines of rats.

Treatment	Niclosamide	Control	<u>U. macrocarpa</u>	<u>A. catechu</u> and <u>C. pepo</u>
Mean dry weight of adult <u>H. diminuta</u>	-0.0	0.26	0.28	0.35
N =	5	5	6	6

(b) Experimental design:

The original experimental design consisted of 4 replications in each of the 4 treatment groups : control, group treated with A. catechu with C. pepo, U. macrocarpa, and a mixture of A. catechu, C. pepo, and U. macrocarpa. Two rats in the control group did not take the infection, as indicated by the negative faecal egg-counts from day 18 to day 28 post-infection. The modified experimental design is shown in Table 29.

(c) Materials and methods:

(i) Infection of the rats

The rats were infected with 10 cysticercoids instead of 5. The rats were infected as described in Experiment 7.

(ii) Preparation of the herbs

The preparations of A. catechu with C. pepo, and U. macrocarpa were as described in Experiment 7. A mixture of A. catechu, C. pepo, and U. macrocarpa was made by mixing equal volumes of the two. The mixture was freshly prepared daily prior to the chemotherapeutic treatment.

(iii) Chemotherapeutic trials and evaluation

The rats were fasted 6 hours before sacrificing them. The cestodes were recovered and dried as described in Experiment 7.

(d) Results:

A. catechu with C. pepo, U. macrocarpa, and the mixture, resulted in 5.56%, -11.11%, and 5.56% reduction of adult cestodes

Table 29: Experiment 8 - Experimental design.

Treatment	Control	<u>A. catechu</u> and <u>C. pepo</u>	<u>U. macrocarpa</u>	<u>A. catechu</u> , <u>C. pepo</u> and <u>U. macrocarpa</u>
No. of replications (rats)	2	4	4	4

(Table 30, Figure 19, Appendix 17). The % reductions of the adult cestodes by U. macrocarpa for Experiment 7 and 8 were similar. The preliminary ANOVA of this experiment was not significant. The a priori testings were also not significant. Thus, none of the treatments had any anthelmintic effect. The three herbs were not synergistic.

The dry weight of *H. diminuta* was reduced by 0.63%, -10.63%, and -16.25% by A. catechu with C. pepo, U. macrocarpa, and the mixture (Table 30, Figure 20, Appendix 18). Both the preliminary ANOVA and the a priori testing,

$$F_s = \frac{MS \text{ (control versus treatment)}}{MS_{\text{within}}} = 0.047$$

$$F_s = \frac{MS \text{ (control versus mixture)}}{MS_{\text{within}}} = 0.112$$

$$\text{where } F_{.05} (1, 10) = 4.96,$$

concluded that the treatment groups were not significantly different. The conclusions agreed with that obtained with the number of cestodes recovered.

A. catechu and C. pepo are reputed for their anticestodal effects. Since they did not seem to elicit an obvious positive response, it was postulated that the concentration may not have been sufficiently high to cause an anthelmintic effect.

3) Experiment 9

(a) Rationale:

A. catechu was selected since arecoline and various other

Table 30: Experiment 8 - The mean number and dry weight
of adult H. diminuta recovered from the intes-
tines of rats treated with combinations of
A. catechu and C. pepo, and U. macrocarpa
(± 95% confidence limits and ± standard error)
and % efficacy.

Treatment	Control	<u>A. catechu</u> and <u>C. pepo</u>	<u>U. macrocarpa</u>	<u>A. catechu, C. pepo,</u> and <u>U. macrocarpa</u>
Mean no. of adult <u>H. diminuta</u>				
+ 95% confidence limits	4.5 $\pm$ 19.1	4.3 $\pm$ 0.8	5.0 $\pm$ 2.3	4.3 $\pm$ 5.3
+ standard error	4.5 $\pm$ 1.5	4.3 $\pm$ 0.3	5.0 $\pm$ 0.7	4.3 $\pm$ 1.7
N =	2	4	4	4
% efficacy	0.0	5.6	- 11.1	5.6
Mean dry weight of adult <u>H. diminuta</u>				
+ 95% confidence limits	0.40 $\pm$ 1.27	0.40 $\pm$ 0.24	0.44 $\pm$ 0.14	0.47 $\pm$ 0.53
+ standard error	0.40 $\pm$ 0.10	0.40 $\pm$ 0.08	0.44 $\pm$ 0.04	0.47 $\pm$ 0.17
N =	2	4	4	4
% efficacy	0.0	0.6	- 10.6	- 16.3

Figure 19: Experiment 8 - The effect of combinations of A. catechu and C. pepo, and U. macrocarpa on the mean number of adult H. diminuta recovered from the intestines of rats $\pm$ 95% confidence limits and $\pm$ standard error.

(Two rats in the control group did not develop the infection).

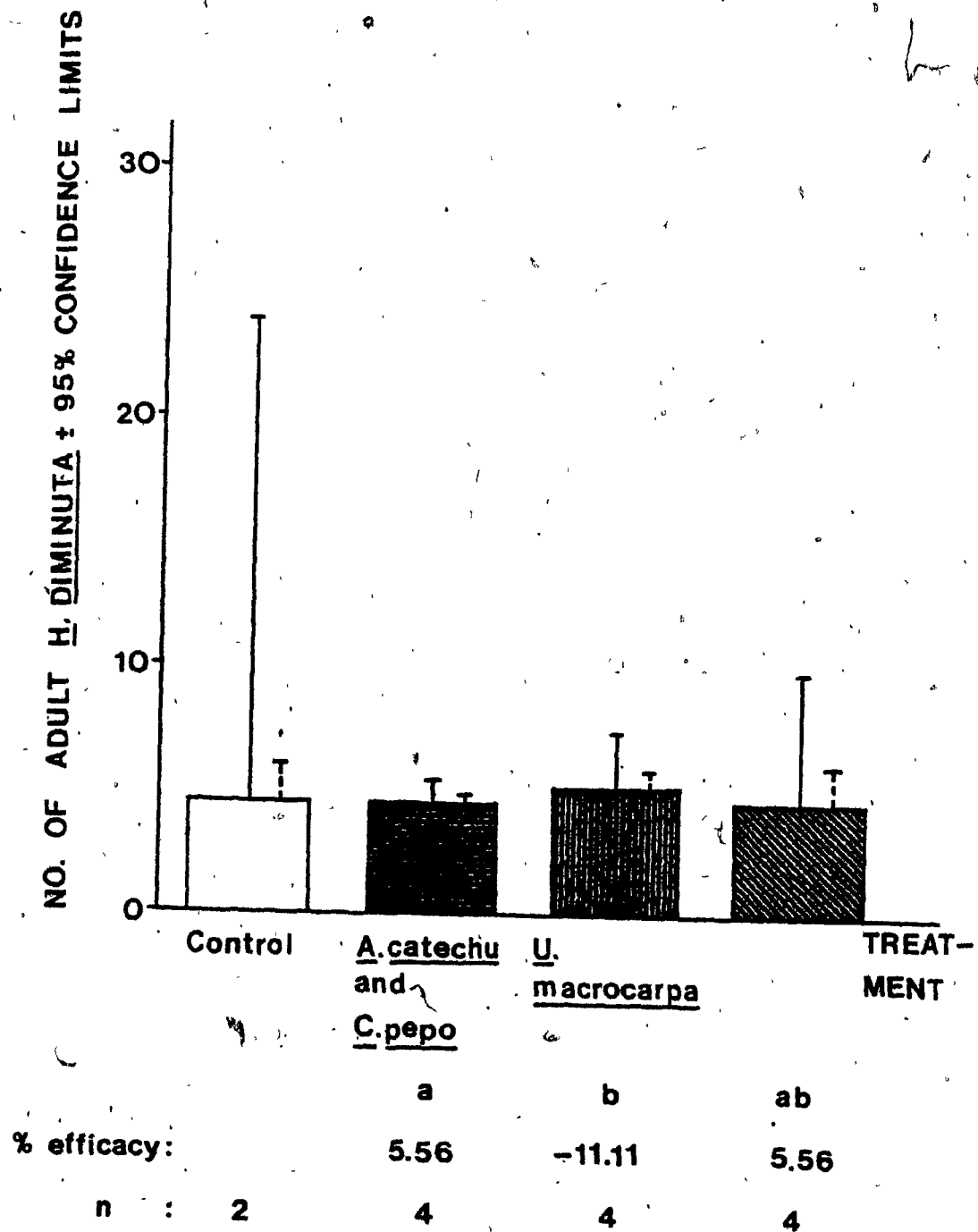
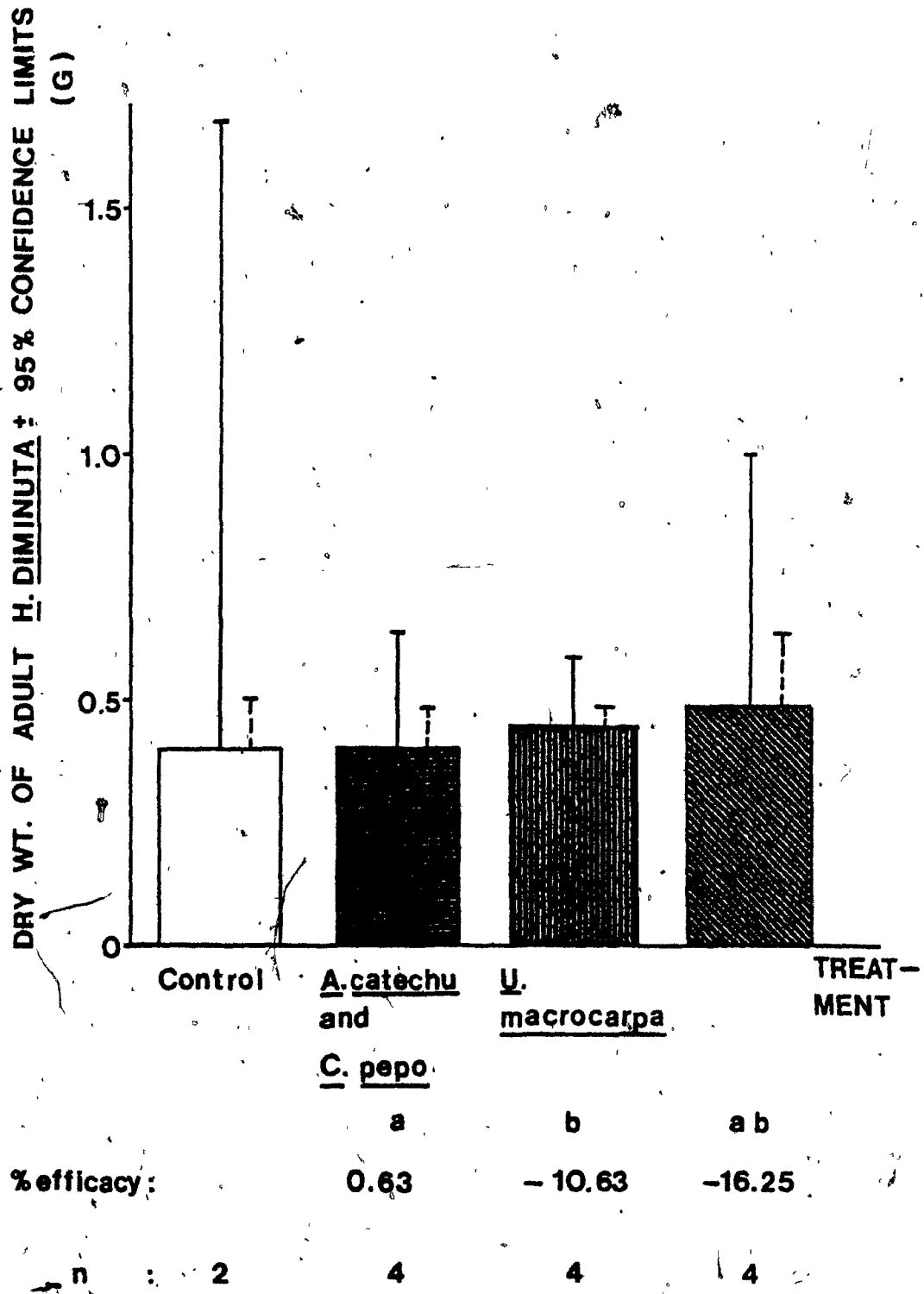


Figure 20: Experiment 8 - The effect of combinations of A. catechu and C. pepo, and U. macrocarpa on the mean dry weight of adult H. diminuta recovered from the intestines of rats $\pm$ 95% confidence limits and $\pm$ standard error.
(Two rats in the control group did not develop the infection).



alkaloids had been extracted from the raw plant material (windholz, 1978). The experiment was set up to compare the effects of different concentrations of A. catechu and arecoline hydrobromide. In this experiment, only A. catechu was used instead of the usual combination, in view of determining the equivalent concentration of raw herbal extract to that of the purified arecoline hydrobromide.

(b) Experimental design:

Six treatment groups were set up. The control had 3 replications. The 3 concentrations of A. catechu were 1.6 g A. catechu/ml, 3.2 g A. catechu/ml, and 6.4 g A. catechu/ml. The numbers of replications in each group were 3, 3, and 4, respectively. In the course of the experiments, 1 rat from the group treated with 1.6 g A. catechu/ml died because of its oversensitivity to ether, after 1 treatment. Two concentrations of arecoline hydrobromide, 6.1×10^{-4} g/(g wt rat) (dose calculated from that suggested by Batham, 1949) and 12.3×10^{-4} g/(g wt rat), were tested. The numbers of replications were 3 and 4, respectively. The actual experimental design is tabulated in Table 31.

(c) Materials and methods:

(i) Infection of rats

Ten week-old rats, weighing from 279.0 g to 328.0 g were each infected per os with ten 17-day-old cysticercoids, dissected from T. confusum, as described in Experiment 7.

(ii) Preparation of A. catechu

A. catechu is included in numerous herbal anthelmintic

Table 31: Experiment 9 - Experimental design.

Treatment	Control	Concentration of <u>A. catechu</u> (g/ml)			Concentration of arecoline hydrobromide (g/(g wt rat))	
		1.6	3.2	6.4	6.1×10^{-4}	12.3×10^{-4}
No. of replications (rats)	3	2	3	4	3	4

prescriptions (Chao, 1940; Chung and Ko, 1976; Hoeppli, 1959; Keys, 1976). The weight of A. catechu prescribed ranged from 1.872 g to 200 g. Since this range was so wide, other limiting factors had to be considered. The concentration for human dose selected was 80 g A. catechu per 250 ml (equivalent to 0.32 g A. catechu/ml). The concentrations to be tested were 5, 10, and 20 times that of human dose, resulting in concentrations of 1.6 g/ml, 3.2 g/ml, and 6.4 g/ml, respectively. The highest concentration was prepared and it was diluted to the required concentration on the day of the treatment.

Pounded A. catechu (480 g) were wrapped in cotton gauze and suspended in a covered clay pot. A volume of 450 ml water was initially boiled. After 1 1/2 hours, 150 ml distilled water were added. It was further heated until 75 ml was obtained. The temperature of the boiling decoction was about 102°C.

(iii) Chemotherapeutic trials

The rats were weighed daily at 09.30 hour. After 18 days post-infection, the faecal egg counts were positive. The rats in the groups treated with A. catechu were given the chemotherapeutic treatment on days 20, 21, and 22 post-infection, while the groups treated with arecoline hydrobromide were administered the drug only on day 20 post-infection. On days 21 and 22 post-infection, they were fed water as was done with the control group on days 20, 21, and 22. The chemotherapeutic treatment was given between 12.00 hour and 13.00 hour, after fasting the animals for 6 hours. The animals had to be lightly etherized as this batch of animals was hyperactive.

(iv) Evaluation of the chemotherapeutic treatment

The rats were observed immediately after the chemotherapeutic treatments. The reactions of the rats were noted.

On day 21 post-treatment, the rats were fasted for 6 hours prior to sacrifice. The cestodes were recovered as described in Experiment 7 and the dry weights were recorded.

(d) Results:

It was originally planned that the rats would be given a 3-day chemotherapeutic treatment regimen of arecoline hydrobromide but since they exhibited epistaxis, vomiting, and catharsis within 30 minutes after treatment, they were given only 1 dose. The rats, however, recovered rapidly from those effects, despite the drastic initial reactions.

One of the rats treated with 1.6 g A. catechu/ml died prior to the second treatment. This was apparently not due to the toxicity of A. catechu. On dissection, 9 cestodes were recovered and the dry weight was 0.91 g. When compared with the results obtained from the rats given 3 treatments, it seemed that the efficacy of 1.6 g A. catechu/ml was not proportional to the frequency of treatment. The data obtained from this rat was not included in Table 32, Figures 21, 22, 23, and 24, and Appendices 19 and 20.

The % reduction of adult cestodes in the following treatment groups, 1.6 g A. catechu/ml, 3.2 g/ml, 6.4 g/ml, 6.1×10^{-4} g arecoline hydrobromide/(g wt rat), and 12.3×10^{-4} g/(g wt rat), were 9.09%, -4.55%, 14.77%, 100%, and 79.55%.

The relationship between the number of adult H. diminuta and

Table 32: Experiment 9 - The mean number of adult
H. diminuta recovered from the intestines of
rats treated with different concentrations of
A. catechu and arecoline hydrobromide, and
% efficacy.

Treatment	Control	Concentration of <u>A. catechu</u> (g/ml)			Concentration of arecoline hydrobromide (g/(g wt rat))	
		1.6	3.2	6.4	6.1×10^{-4}	12.3×10^{-4}
Mean no. of adult <u>H. diminuta</u>						
+ 95% confidence limits	7.3 ± 5.2	8.0 ± 12.7	7.7 ± 3.8	6.3 ± 2.7	0.0 ± 0.0	1.5 ± 4.8
+ standard error	7.3 ± 1.2	8.0 ± 1.0	7.7 ± 2.7	6.3 ± 0.9	0.0 ± 0.0	1.5 ± 1.5
N =	3	2	3	4	3	4
% efficacy	0.0	- 9.1	- 4.6	14.8	100.0	79.6
Mean dry weight of adult <u>H. diminuta</u>						
+ 95% confidence limits	0.38 ± 0.27	0.71 ± 0.57	0.49 ± 0.24	0.42 ± 0.23	0.00 ± 0.00	0.14 ± 0.43
+ standard error	0.38 ± 0.06	0.71 ± 0.04	0.49 ± 0.06	0.42 ± 0.07	0.00 ± 0.00	0.14 ± 0.14
N =	3	2	3	4	3	4
% efficacy	0.0	- 83.9	- 27.8	- 9.6	100.0	63.8

Figure 21: Experiment 9 - The effect of different concentrations of A. catechu and arecoline hydrobromide on the mean number of adult H. diminuta recovered from the intestines of rats $\pm$ 95% confidence limits and $\pm$ standard error.

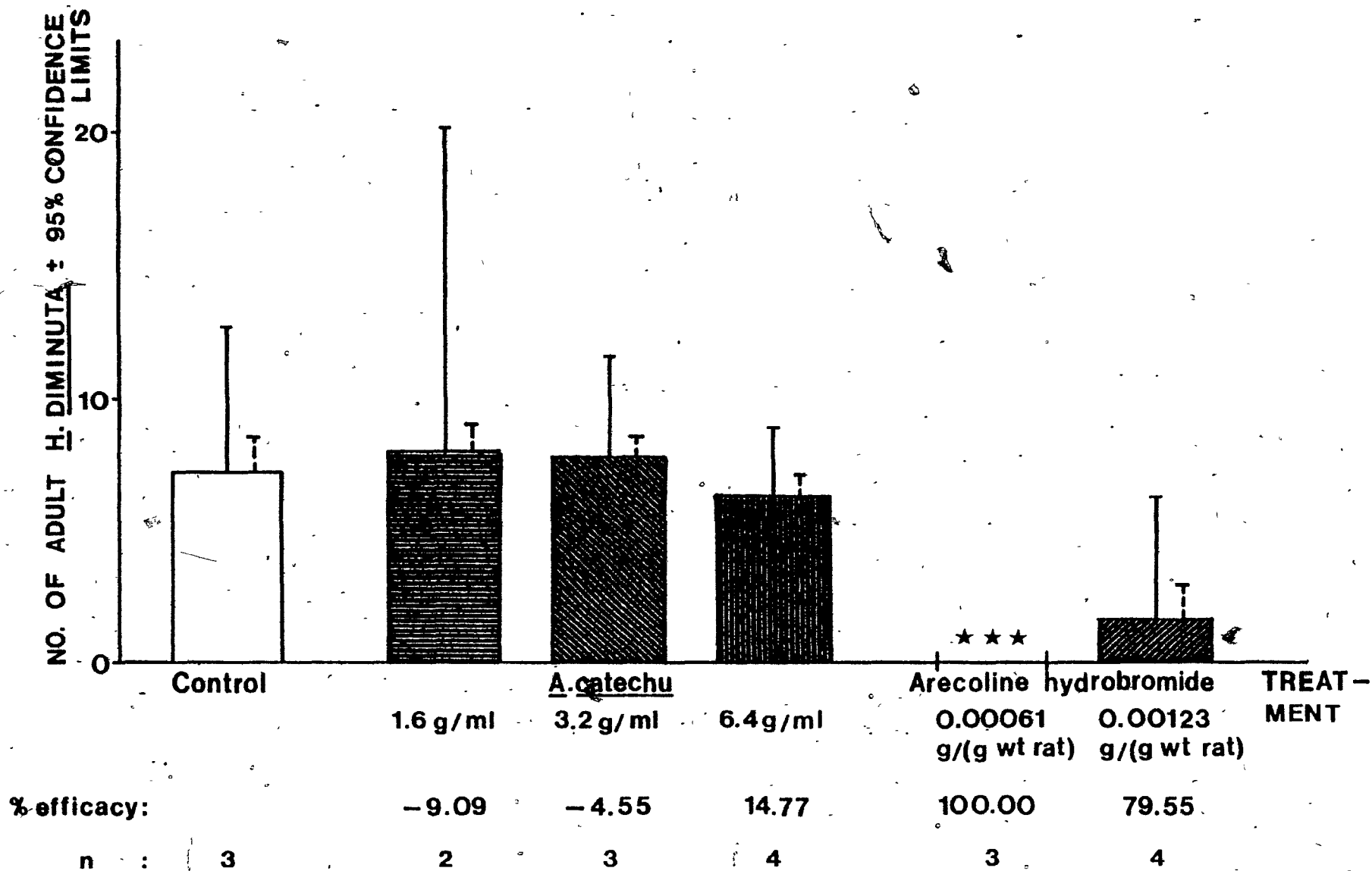
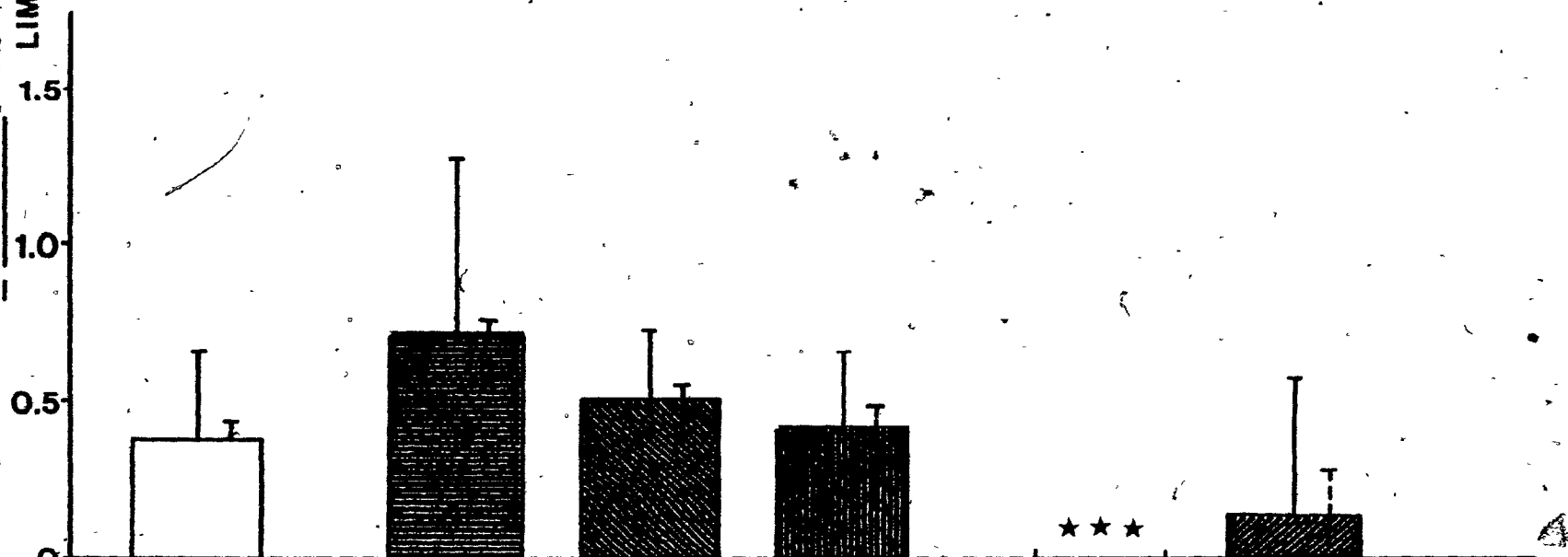


Figure 22: Experiment 9 - The effect of different concentrations of A. catechu and arecoline hydrobromide on the mean dry weight of adult H. dimi-
muta recovered from the intestines of rats
± 95% confidence limits and ± standard error.

DRY WT. OF ADULT H. DIMINUTA $\pm$ 95% CONFIDENCE LIMITS (G)



% efficacy:

-83.91

-27.83

-9.57

100.00

63.78

n : 3

2

3

4

3

4

TREAT-
MENT

Figure 23: Experiment 9 - A plot of the mean number of
adult H. diminuta recovered from the intestines
of rats against the concentration of A. catechu.
(The straight line was fitted by eye).

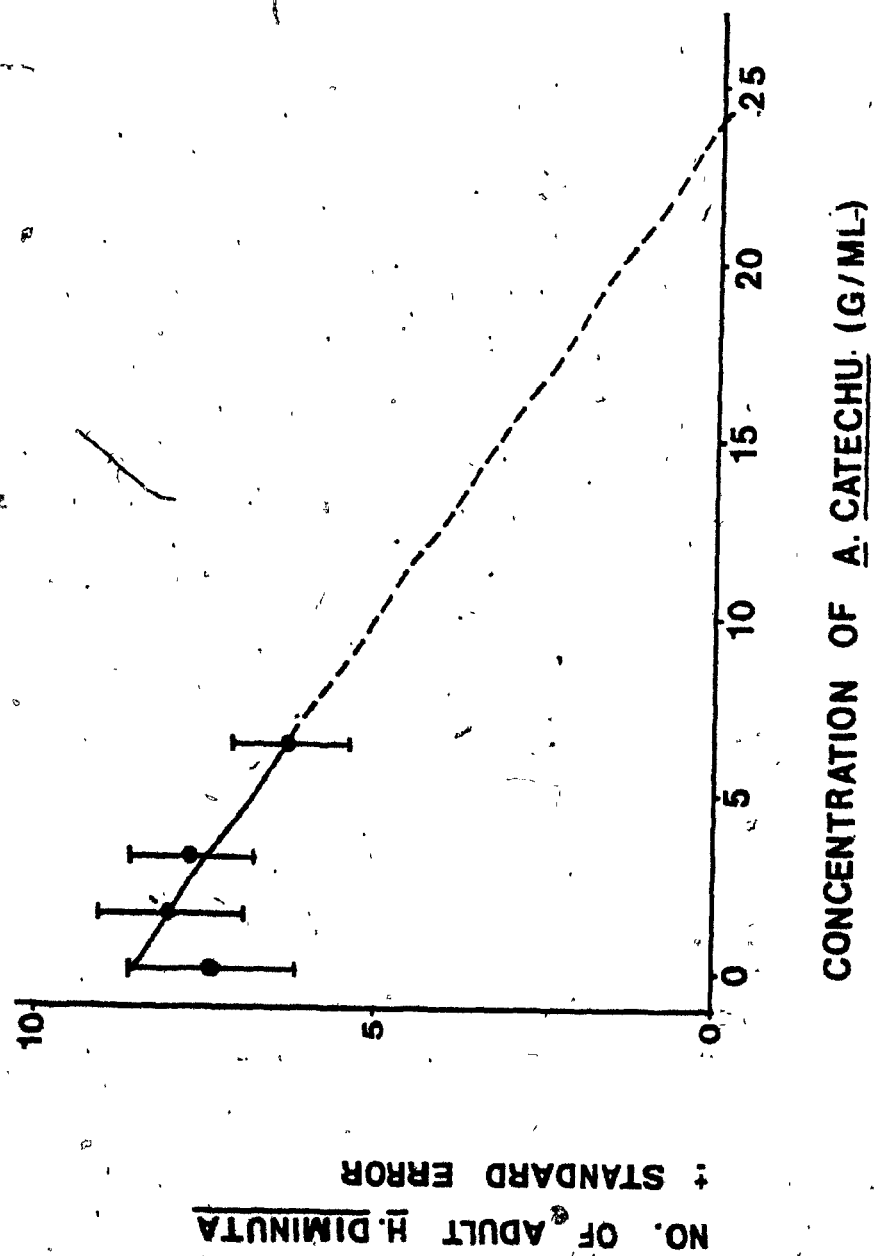
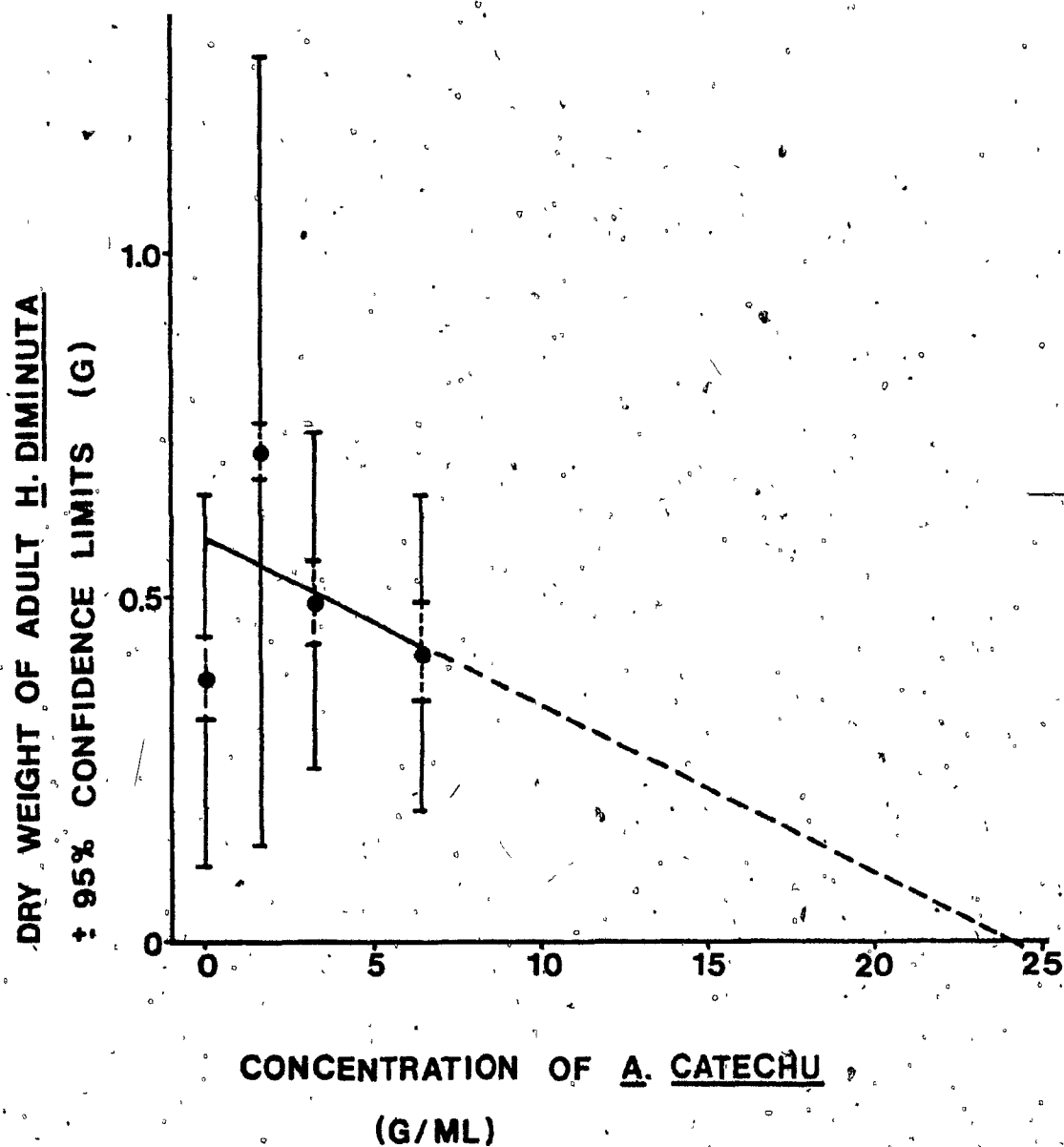


Figure 2h: Experiment 9 - A plot of the mean weight of
adult H. diminuta recovered from the intestines
of rats against the concentration of A. catechu.
(The straight line was fitted by eye).



the concentration of A. catechu can be described by this straight line equation (Figure 23) :

$$Y + 0.07 X = 8.6$$

where Y = number of adult H. diminuta recovered from the rats

X = concentration of A. catechu (g/ml).

The preliminary ANOVA indicated that the treatments were significantly different. The a priori tests gave the following results :

$$F_s = \frac{MS \text{ (control versus treatment)}}{MS_{\text{within}}} = 5.63^*$$

$$F_s = \frac{MS \text{ (control versus herbal treatment)}}{MS_{\text{within}}} = 0.03$$

$$F_s = \frac{MS \text{ (herbal treatment versus arecoline hydrobromide)}}{MS_{\text{within}}}$$

$$= 39.19^{***}$$

$$\text{where } F_{.05} (1, 19) = 4.38$$

$$F_{.01} (1, 19) = 8.19$$

$$F_{.001} (1, 19) = 15.10$$

which could mean that arecoline hydrobromide contributed to the significant difference. The a posteriori comparison is shown in Table 33.

The conclusions are similar to that of the a priori testing.

The % efficacies calculated from the dry weights were -83.91%, -27.83%, -9.57%, 100%, and 63.78% for the treatment groups, 1.6 g A. catechu/ml, 3.2 g A. catechu/ml, 6.4 g A. catechu/ml, 6.1×10^{-4} g arecoline hydrobromide/(g wt rat), and 12.3 g arecoline hydrobromide/

Table 33: Experiment 9 - A posteriori testing of the mean
number of adult H. diminuta recovered from the
intestines of rats, using the SNK procedure.

Treatment.	Concentration of arecoline hydrobromide (g/(g wt rat)) 6.1 X 10 ⁻⁴ 12 X 10 ⁻⁴	6.4 g <u>A.c.</u> /ml	Control	3.2 g <u>A.c.</u> /ml	1.6 g <u>A.c.</u> /ml
Mean no. of adult <u>H. diminuta</u> N =	0.00 1.50	6.25	7.33	7.67	8.00
	3 4	4	3	3	2

(g wt rat), in that order (Table 32, Figures 22 and 24, and Appendix 20).

A plot of the dry weight of adult H. diminuta recovered from the rats (Y -axis) against the concentration of A. catechu (X - axis) gave a linear relationship (Figure 24) :

$$Y + 0.03X = 0.59$$

The preliminary ANOVA showed that the effects of the different treatments were significant ($P < .01$). The a priori testing gave the following :

$$(i) F_s = \frac{MS \text{ (control versus treatment)}}{MS_{\text{within}}} = 0.42$$

This is not significant since $F_{.05} (1, 13) = 4.67$

$$(ii) F_s = \frac{MS \text{ (A. catechu versus arecoline hydrobromide)}}{MS_{\text{within}}} = 28.79 ***$$

This is significant since $F_{.001} (1, 13) = 17.8$.

This would imply that the negative effects of A. catechu and the positive results of arecoline hydrobromide must have balanced each other out to give a non-significance test in (i).

$$(iii) F_s = \frac{MS \text{ (control versus A. catechu)}}{MS_{\text{within}}} = 1.36$$

The anthelmintic effect of A. catechu is not significant.

$$\text{iv) } F_s = \frac{MS(\text{among } \underline{A. catechu})}{MS_{\text{within}}} = 2.17$$

$$F_{.05}(2, 13) = 3.81$$

The concentrations of A. catechu did not seem to have a proportional effect that is significantly different.

The results of the a posteriori testing are summarized in Table 34. The control group was not significantly different from all the groups treated with A. catechu but was significantly different ($P < .05$) from that treated with 6.1×10^{-4} g arecoline hydrobromide/(g wt rat). The groups treated with arecoline hydrobromide were not significantly different from each other, indicating that 6.1×10^{-4} g arecoline hydrobromide/(g wt rat) was sufficient to elicit a positive anthelmintic response. It also showed that the anthelmintic effect of the two concentrations are not significantly different. The three groups treated with A. catechu were also not significantly different from each other. The group treated with 1.6 g A. catechu/ml, however, was significantly different from both groups treated with arecoline hydrobromide. Larger sample sizes would probably detect the anthelmintic properties of A. catechu.

Table 34: Experiment 9 - A posteriori testing of the
mean dry weight of adult H. diminuta recovered
from the intestines of rats, using the SNK
procedure.

Treatment	Concentration of arecoline hydrobromide (g/(g wt rat))		Control	Concentration of <u>A. catechu</u> (g/ml)		
	6.1×10^{-4}	12.3×10^{-4}		6.4	3.2	1.6
Mean dry weight of adult <u>H. diminuta</u>	0.00	0.14	0.38	0.42	0.49	0.71
N =	3	4	3	4	3	2

II) In vitro experiment

1) Experiment 10

(a) Rationale:

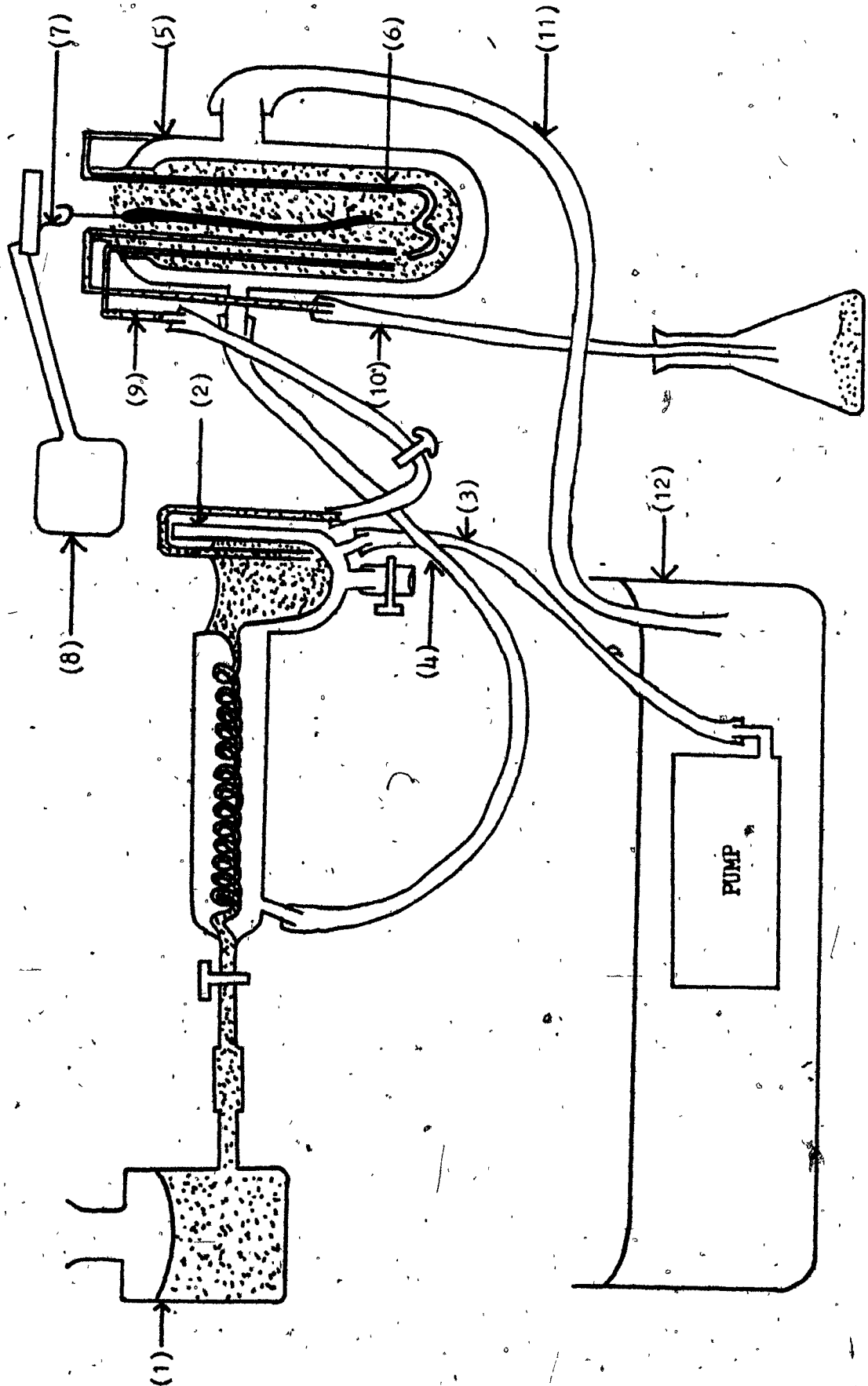
An in vitro experiment was designed to supplement the in vivo experiments. Attempts were made to experiment with N. brasiliensis and H. diminuta, using the Grass Model 7 Polygraph but it was not sufficiently sensitive. A. lumbricoides was used instead since it was readily available from abattoirs in Montreal, and was much easier to handle.

(b) Materials and methods:

(i) The apparatus

Figure 25 shows the apparatus used. The glass jar (1) supplied the artificial perienteric fluid (APF) (Appendix 21 for the components). Organ Bath A (2) maintained a supply of APF at about 36°C - 37°C. The rubber tubing (3) connected the pump to the Organ Bath A, generating a flow of water at about 37°C - 38°C. The rubber tubing (4) joined Organ Bath A to Organ Bath B (5). (Plate 5). The glass rod support (6) weighed down the tail end of A. lumbricoides, thus positioning it. Glassware was used throughout since metallic supports and tubes could be toxic to the parasite. The force-displacement transducer (7) was attached to the anterior end of A. lumbricoides. The transducer was also connected to the Grass Model Polygraph (8) (Plate 6). A glass tubing (9) supplied Organ Bath B with warm APF while (10) was used for draining. The rubber tubing (11) recycled the water back to

Figure 25: Apparatus for Experiment 10.



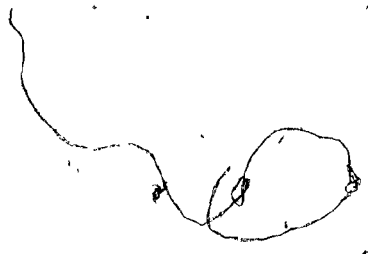


Plate 5: Organ Bath B (see pp.189), with A. lumbricoides
in artificial perienteric fluid.



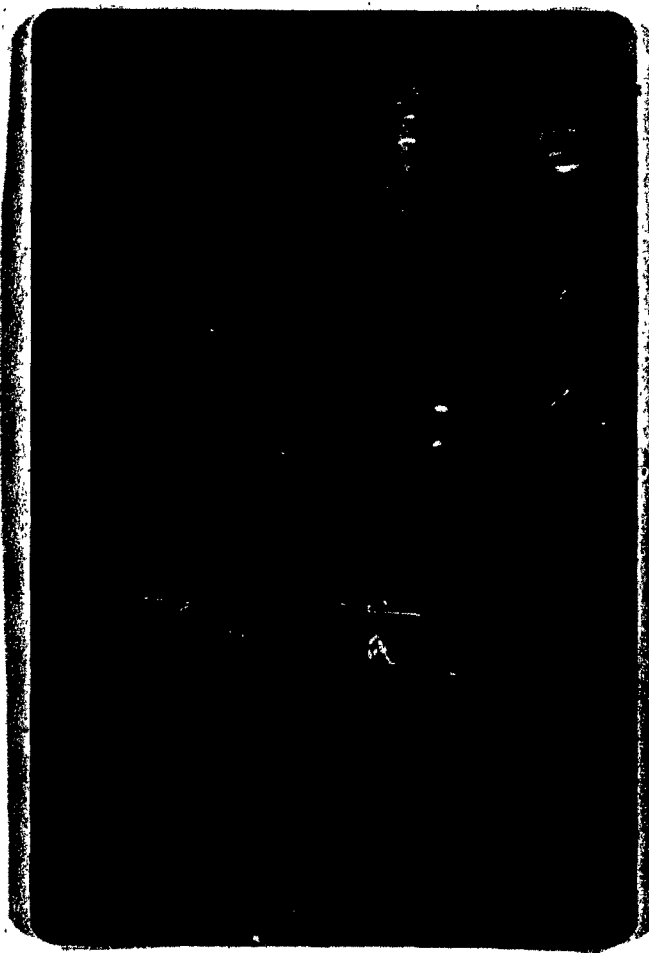
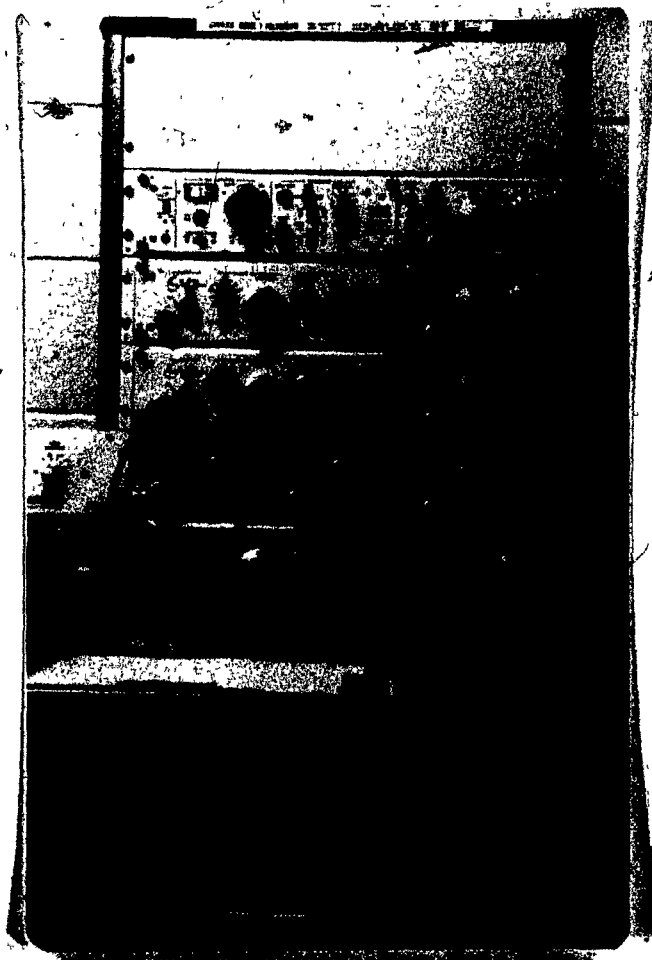


Plate 6: Grass Model 7 Polygraph.



the water bath (12).

(ii) The operation of Grass Model 7 Polygraph

The Grass Model 7 Polygraph was kindly loaned by Dr. P. C. Laue of the Department of Animal Science, Macdonald College.

The operation of the machine, the calibration of the driver amplifier, and the connection of the transducer were carried out as described in the instruction manual (Appendix 22).

The chart speed control knob was set at 0.5mm/s. The transducer was calibrated by hanging gram weights suspended on very light thread, immediately after experimenting with each parasite.

(iii) The collection, storage, and selection of the parasite

A. lumbricoides were obtained from an abattoir in Montreal. They were kept in physiological saline during the transportation.

On arrival at the laboratory, the worms were kept in an incubator at 37°C.

Male worms of length, ranging from 14 - 18 cm were selected and transferred to a large 2 litre conical flask filled with APF. The parasites were kept in a water bath at 37°C - 38°C. The solution was changed daily or as often as necessary. Although the worms could survive as long as 5 days, the oldest specimens used had been kept under such in vitro conditions for only 3 days.

The specimens selected for the experiment were pink and active at 37°C.

(iv) The drugs used

Q. indica was prepared as described in Experiment 5 and

diluted to 0.45 g Q. indica/(ml APF); piperazine citrate, U.S.P. - Antepar^R (Burroughs Wellcome and Co. (Canada) Ltd. Montreal; Lot 395F), diluted to 50 mg piperazine hexahydrate/(ml APF); pyrantel pamoate, Combantrin^R (Pfizer Company Ltd, Montreal; Lot 702 18041), diluted to 72 mg pyrantel pamoate/(ml APF).

(v) The testing of the drugs

The parasite moved freely in APF for 30 minutes. Three ml APF was then removed using a calibrated syringe connected to a glass tubing (9). An equal volume of Q. indica was added. The contractions of the parasite were recorded for 30 minutes. Three rinsings with APF were performed. After 30 minutes of normalization in APF, 3 ml of APF were removed and replaced with piperazine citrate. The effect of piperazine citrate was noted for 30 minutes before washing with warm APF was repeated thrice. The movement of the parasite was allowed to stabilize for 30 minutes before 3 ml of APF were removed. An equal volume of pyrantel pamoate was assayed. The recording continued for 30 minutes before rinsing was repeated thrice. A. lumbricoides was left in APF for 30 minutes to note whether the action of pyrantel pamoate was reversible.

Organ Bath B was filled up to 220 ml. The effective concentration (EC) of the drugs were calculated by the following formula :

$$EC = \frac{\text{Concentration of drug used} \times \text{Volume of drug used}}{220}$$

(c) Results:

Preliminary results indicated that Q. indica had no obvious effect on A. lumbricoides over a time period of 30 minutes. The effect of piperazine citrate alone was slow when compared with that of pyrantel pamoate. The immobilization effect of piperazine, as indicated by the decrease of frequency and amplitude of peaks, began about 30 minutes after the assay of 2.272×10^3 ug piperazine citrate/ml while paralysis by 9.82×10^2 ug pyrantel pamoate/ml usually occurred about 5 minutes after its addition.

The results of the testing of the drugs are shown in Tables 35 and 36, and Figures 26 and 27. Only two of the representative physiographs are shown in Figure 28 as the others are similar. The physiographs showed that 6.136×10^3 ug Q. indica/ml had no obvious qualitative or quantitative effect on A. lumbricoides after 30 minutes. The contractions normalized after rinsing with warm APF.

The effect of 681 ug piperazine citrate/ml was also not obvious after 30 minutes. This could either be interpreted as piperazine citrate having an insignificant effect over a period of 30 minutes or as Q. indica having an antagonistic effect on piperazine citrate, thus delaying its action. Pyrantel pamoate at a concentration of 982 ug/ml paralysed A. lumbricoides in 4.3 ± 0.9 minutes (Table 35). This effect could be due to a cumulative effect of Q. indica, piperazine citrate, and pyrantel pamoate. Aubry, Cowell, Davey, and Shevde (1970) determined the times taken to paralyze male worms with doses of 25 ug/ml and 100 ug/ml to be 49.4 ± 33.4 minutes and 23.6 ± 12.5 minutes. When the values were plotted on a semilog scale, a straight line (Figure 27), $Y + 24.0 X = 76.0$, where $Y = \log$, concentration of pyran-

Table 35: Experiment 10 - The time for 982 ug pyrantel
pamoate/ml to paralyse A. lumbricoides (mins
 $\pm$ 95% confidence limits and $\pm$ standard error)

Parasite No.	Time for 982 μ g pyrantel pamoate to paralyse <u>A. lumbricoides</u>	
	Equivalent in length (mm) ¹	Equivalent in minutes
1	80	2.7
2	150	5.0
3	155	5.2
4	130	4.3
5	80	2.7
6	110	3.6
7	145	4.8
8	190	6.3
9	155	5.2
10	100	3.3
ΣY	1295	43.1
$\bar{Y}$	129.5	4.31
$\pm 95\%$ confidence limits		0.858
$\pm$ standard error		0.380

1. The chart speed was 0.5 mm/s.

Table 36: Experiment 10 - The effect of different concentrations of pyrantel pamoate on the time to paralyse A. lumbricoides.

Concentration of pyrantel pamoate (ug/ml)	25	100	982
Log ₁₀ concentration	1.398	2.000	2.992
Time to paralyse <u>A. lumbricoides</u> (mins.)	49.4 ¹	23.6 ¹	4.3
+ 95% confidence limits	± 33.4	± 12.5	± 0.9
+ standard error	± 12.0	± 4.5	± 0.4
N =	5	5	10

1. Values were obtained from Aubry, M.L., Cowell, P., Davey, M.J., and Shevde, S. (1970). Aspects of the pharmacology of a new anthelmintic : pyrantel. British Journal of Pharmacology 38: 332-344.

Figure 26: Experiment 10 - A plot of the time to paralyse A. lumbricoides (mins) against the concentration of pyrantel pamoate (ug/ml).

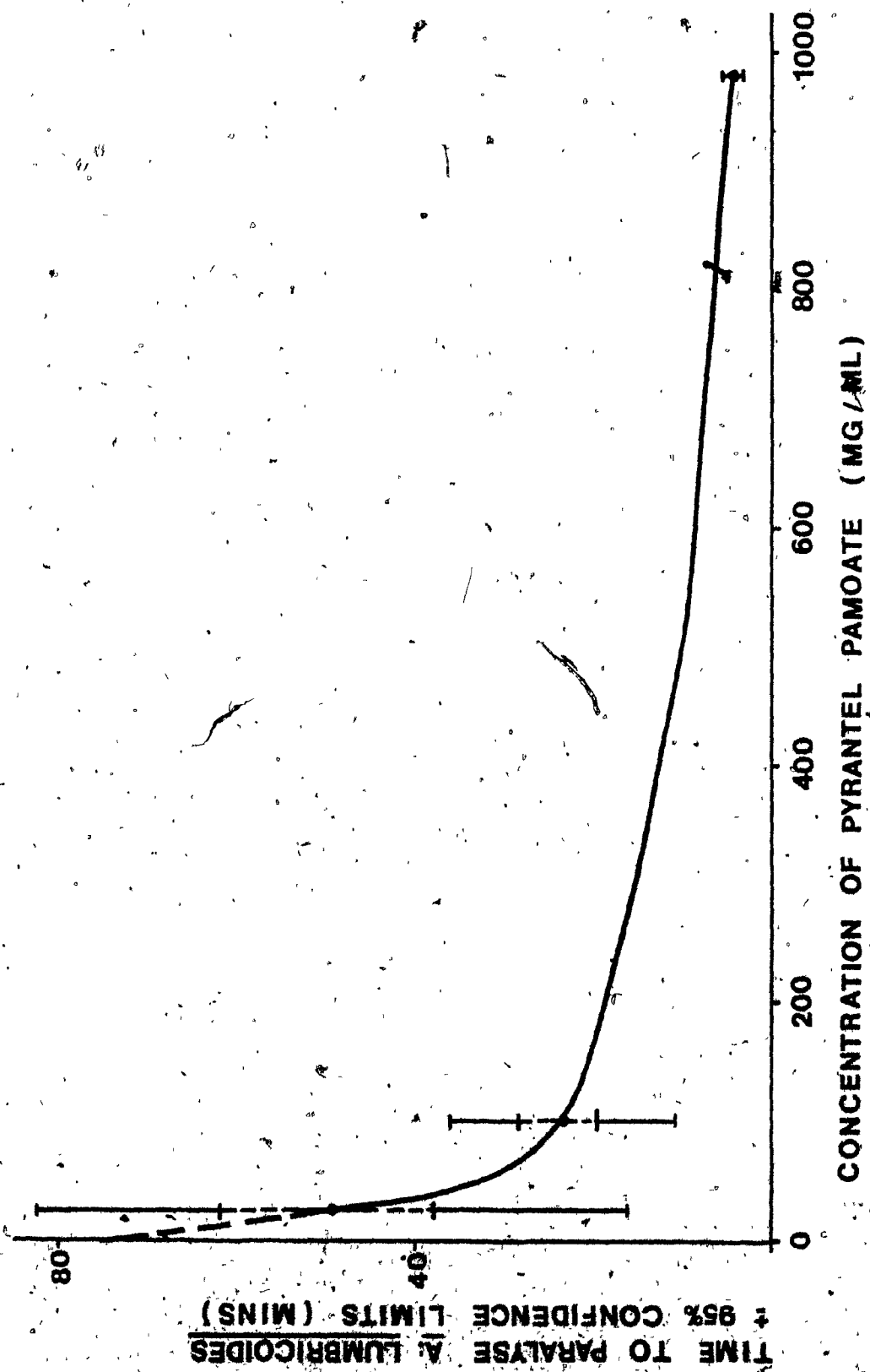
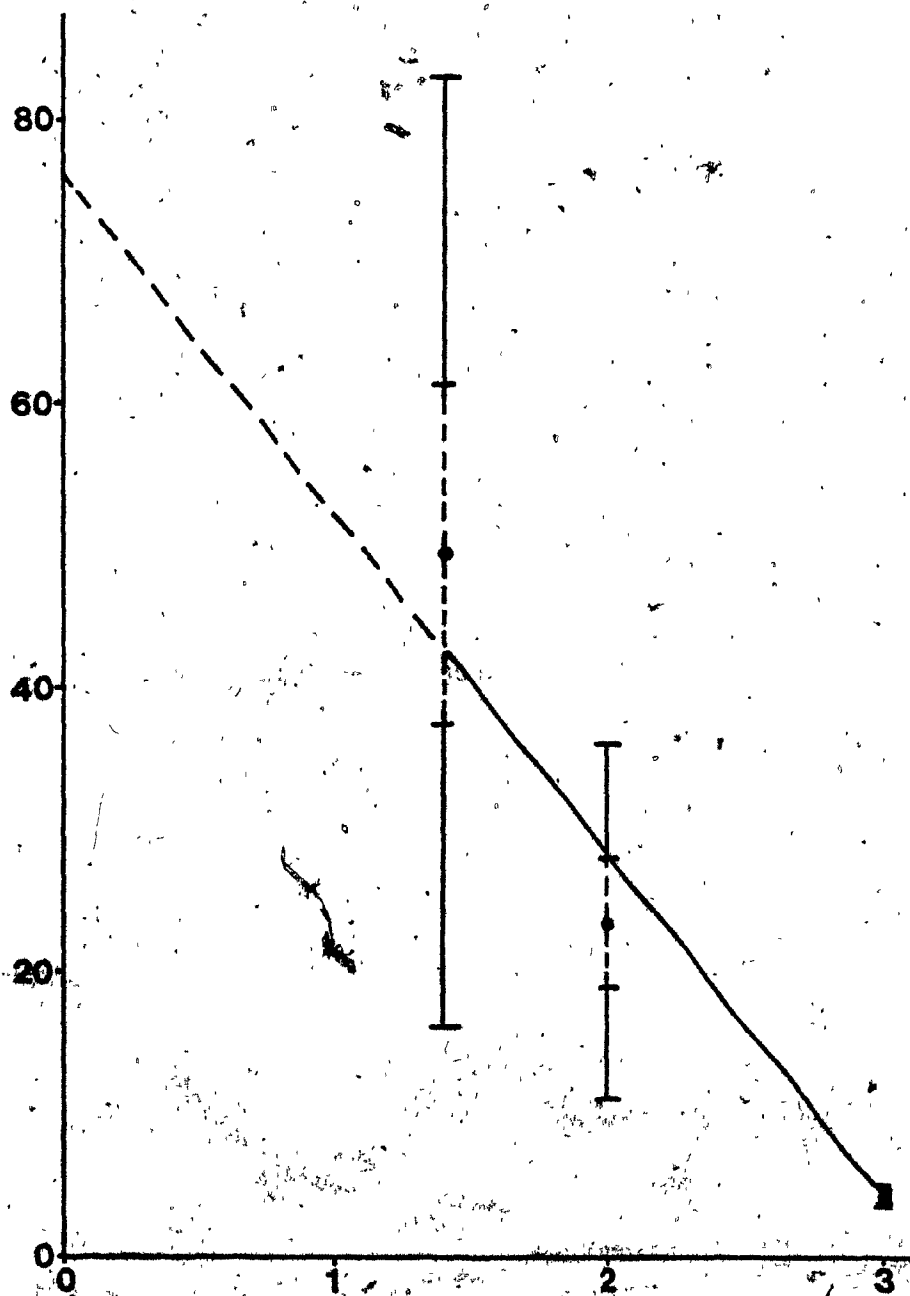


Figure 27: Experiment 10 - A plot of the time to paralyze A. lumbricoides (mins) against the $\log_{10}$ concentration of pyrantel pamoate. (The straight line was fitted by eye).

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LINE 10 BYVFAE V. LUMBRICOIDES

TIME TO PARALYSE A. LUMBRICOIDES $\pm$ 95% CONFIDENCE LIMITS (MINS)

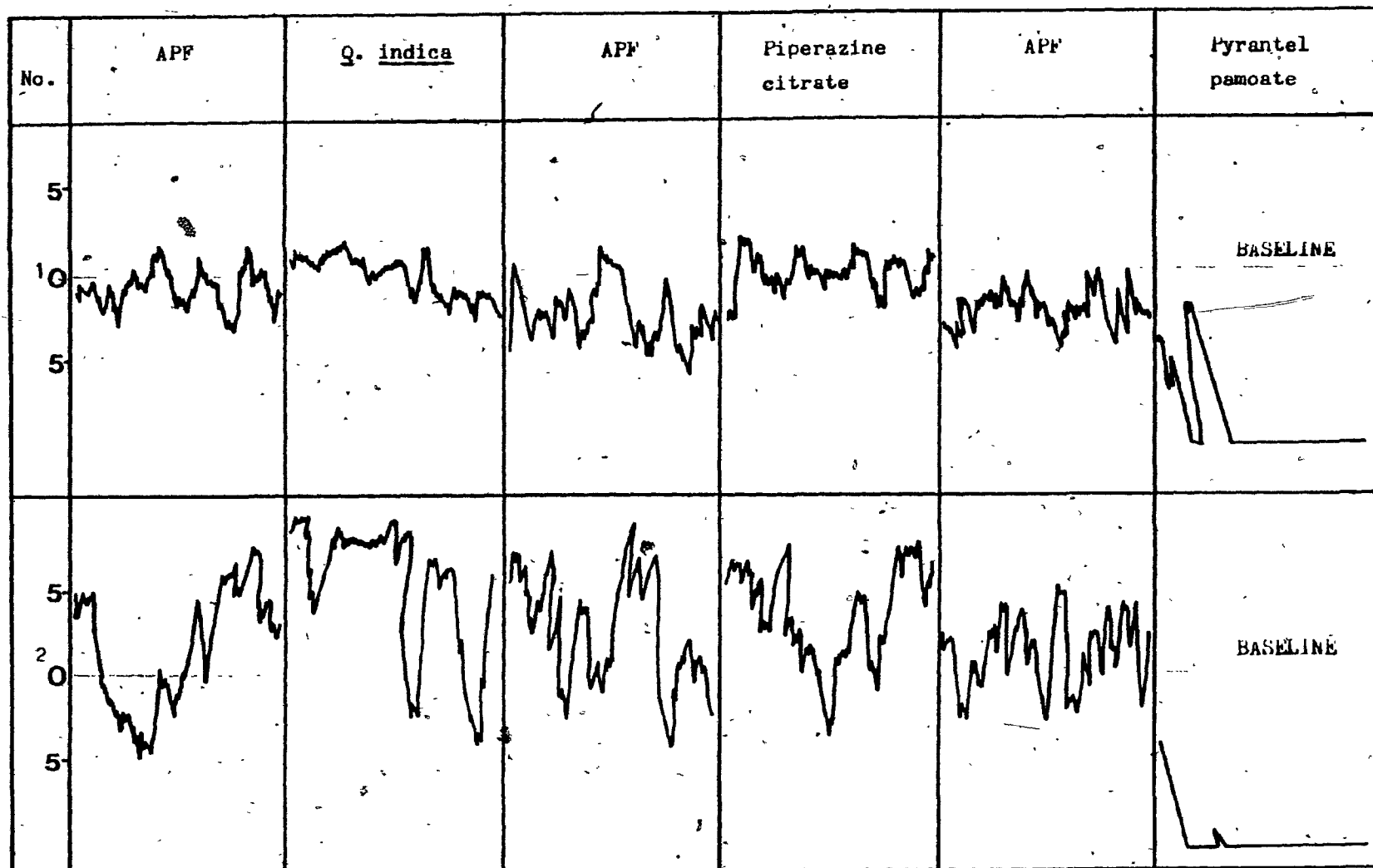


LOG. CONCENTRATION OF PYRANTEL PAMOATE

Figure 28: Experiment 10 - Physiographs of A. lumbricoides.

LOG CONCENTRATION OF RYANETINE

WEIGHT (g)



TIME (0.5 mm is equivalent to 1 s)

tel pamoate and X = time to paralyse A. lumbricoides, was fitted by eye. The effect of pyrantel pamoate was multiplicative. The straight line also implied that the paralytic effect of pyrantel pamoate observed was due only to the effect of pyrantel pamoate and was not due to a cumulative effect since Aubry et al. (1970) worked with single drugs. Besides, the workers noted that piperazine antagonized the effect of pyrantel pamoate, implying that if there were interacting effects, the experimental time taken to paralyse the parasite would have been much longer than that obtained. It is also known that the action of piperazine is reversible.

Discussion

The Scientific Method was applied to the experiments designed by quantifying the data so that the results could be analysed statistically. Consequently, hypotheses could be subjected to falsification, since the Scientific Method depends on the falsifiability of the hypotheses. Variation was reduced by using animals in a similar age group, and within a narrow weight range. The double-blind technique was simulated by ensuring that the person who helped in the feeding of the rats with the chemotherapeutic treatment, did not know the order of the treatment. Randomisation was considered after ensuring that the experimental animal groups were homogeneous. Control groups were used throughout since it was considered as one of the most important concepts in biological experimentation.

The criterion used to evaluate the efficacy of the chemotherapeutic treatment on N. brasiliensis was the % reduction of parasite burden. It was less variable than the daily faecal egg output. It was also more reliable since it implicitly measured the number of parasites killed while a chemotherapeutic treatment could merely suppress the faecal egg output.

The % reduction by the herbal mixture, M. azedarach (seed), M. lapidescens, and Q. indica (Experiment 1), was slightly higher than that obtained by individual herbs (Experiments 2 and 3). This could be attributed to the mild synergistic effect of Q. indica, M. azedarach, and M. lapidescens on each other. The % reductions due to Q. indica were markedly different in Experiments 2, 3, 4, and 5. However, it was observed that the % efficacy of Q. indica was similar in Experiments 2,

5, and 6. Extrapolating from Figure 14, it was found that 0.54 g Q. indica/ml gave a 23.2% reduction of adult N. brasiliensis, which was close to 25.6% obtained in Experiment 2. The method of preparation of Q. indica was similar in Experiments 2, 5, and 6 except for some minor details. The time of extraction was very short for Experiment 3 and the Soxhlet apparatus was used in Experiment 4. It can be concluded that the time of extraction and the method of extraction are crucial. The method of decoction (as in Experiment 2) and infusion (as in Experiments 5 and 6) gave similar results. They were more efficacious than the use of a Soxhlet apparatus (Experiment 4).

A preliminary extraction of M. azedarach (seed), Q. indica, and M. lapidescens (Appendix 23) was kindly performed by Dr. John Devlin of Pharma Research, formerly based at 250, Hymus Boulevard, Point-Claire, Québec. The presence of anthraquinone was detected in Q. indica, as indicated by fluorescence in the ethanol extract. A milder fluorescence was also detected in the ethanol extract of M. azedarach. Active anthelmintic compound could possibly be found in Q. indica and M. azedarach, the candidate compounds, despite the negative results of the in vivo trials in the laboratory rat - N. brasiliensis model.

The discrepancy between the detection of potential anthelmintic substances in Q. indica and M. azedarach (seed) and the low % efficacy in biological testing could be attributed to the method of extraction. This has interesting implications. Over many years, traditional Chinese herbal anthelmintic prescriptions have lost much of the specific instructions for the preparation. The usual instructions consist of making a decoction by boiling the herbs in one and a quarter bowl (about 300 ml) to eight-tenths of it (about 240 ml = 1 bowl). It

is possible that the investigation of the method of optimum extraction could result in a detection of a higher anthelmintic efficacy in most of the herbs. My results at several concentrations and using different methods of preparing Q. indica, support this.

The different infective doses could have affected the absolute number of adult N. brasiliensis recovered but the effect on the % reduction, as compared to the control, would be negligible.

Combinations of herbs reduced the % efficacy of the individual herbs in Experiment 4 but the reduction was not significantly different. Prescriptions including A. catechu, Q. indica, and M. azedarach are found in Chinese medical texts. They, however, do not have synergistic effects on each other. The prescription of various combinations of anthelmintic herbs could be due to the influence of the paradigmatic Chinese medical principle of holism. It could also be rationalized as a broad-spectrum anthelmintic mixture, aiming at multiple infections, rather than single infections.

The difference in the % efficacy of M. azedarach in Experiments 2 and 4 could also be attributed to the different botanical parts used. The seeds of M. azedarach were used in Experiment 2 while the roots were used in Experiment 4. The seeds were also used in Experiment 1, and the % reduction, although higher in Experiment 1 due to possible synergistic effects of the mixture, was in a similar range as that of Experiment 2.

The only herb used in the in vivo bioassay on N. brasiliensis which seemed to show any positive action was Q. indica. Its efficacy, however, is insignificant, compared to pyrantel pamoate. Q. indica, however, is cheaper than pyrantel pamoate as it is commonly grown in tropical countries, where the prevalence of hookworm diseases is high.

There are various other factors that could account for the low % efficacies of the herbal treatments and for the variability observed. The active ingredients are plant products and are, consequently, influenced greatly by edaphic and climatic conditions. The low % efficacies could be due to the absence of essential precursors in the soil for the biosynthesis of the active ingredients. It was not assumed that although the herbs were brought from one particular shop at one time, the herbs of each particular kind came from the same shipment or were grown in the same locality. Pyrantel pamoate and niclosamide gave consistent results. This could not be assumed with herbal treatments because of the various elements of variability described above.

The in vitro testing showed that Q. indica had negligible effects on the motility and muscle tone of A. lumbricoides within a time span of 30 minutes, as compared with pyrantel pamoate, which paralysed A. lumbricoides in about 4 minutes. Preliminary in vitro observations of the effect of Q. indica on N. brasiliensis showed greater motility instead of paralysis. Chen and Hou (1926) reported that Q. indica did not expel any ascarid worms in dogs or in man. Three patients who showed positive faecal egg-count after treatment with Q. indica received oil of chenopodium followed by carbon tetrachloride and a saline cathartic. Negative egg outputs were obtained during the following week. The results obtained in this laboratory evaluation supported the observations of Chen and Hou (1926). Q. indica may not be effective against A. lumbricoides but a high concentration of Q. indica did statistically significantly reduced the number of adult N. brasiliensis recovered (combined results of Experiments 5 and 6).

The anthelmintic effect of M. azedarach (seed) seemed minimal. A. catechu with C. pepo, M. azedarach (root), and M. lapidescens did not seem to have any anthelmintic effect against N. brasiliensis. Their effects against A. lumbricoides and E. vermicularis have yet to be investigated in an in vivo laboratory host - parasite model. This laboratory evaluation clearly shows that the anthelmintic effect of pyrantel pamoate is far greater than that of A. catechu with C. pepo, M. lapidescens, M. azedarach (seed and root), and Q. indica. Although high concentrations of Q. indica had some positive anthelmintic effect, its efficacy is lower than that of pyrantel pamoate. Concentrations higher than 0.450 g Q. indica/ml (equivalent to 10X human dose) would not elicit a proportionally higher efficacy rate since the gradient was greatest between 0.250 g to 0.450 g Q. indica/ml (Figure 16). Its toxicity at concentrations higher than 0.900 g Q. indica/ml (equivalent to 20X human dose) was not investigated. It was not toxic to rats at that concentration.

The two criteria used to evaluate the efficacy of chemotherapeutic treatment on H. diminuta were the number of adult cestodes and the dry weight of the cestodes recovered. The number of cestodes implicitly measured the immediate chemotherapeutic effect while the dry weight also detected the after-effect of the chemotherapeutic treatment on the growth of the cestodes.

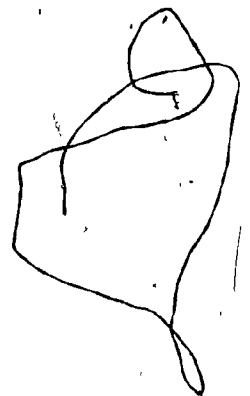
The % reductions of the number of cestodes by A. catechu with C. pepo, U. macrocarpa, and niclosamide were -75.93%, -11.11%, and 100% (Experiment 7). The % efficacies as measured by the dry weight were -33.71%, -6.82%, and 100% in the order mentioned above. The absolute values were different for the two criteria but the trend was

similar. In both evaluations, the effects of the herbal treatments were not significantly different from the controls. In Experiment 8, the expected number of cestodes recovered (4.63) for the group treated with A. catechu, C. pepo, and U. macrocarpa combined, was higher than the observed value (4.25). This may be a synergistic effect of the combination. The preliminary ANOVA and a priori testing of both evaluations, however, showed that the herbal treatments were not significantly different from the controls. In Experiment 9, although the concentration of 6.4 g A. catechu/ml reduced the number of cestodes, the a priori and a posteriori testings did not detect a significant difference.

The dry weight per cestode was inversely proportional to the number of cestodes (Chappell and Pike, 1976) due to intra-specific competition. This could complicate the interpretation of the results on the dry weight of the cestodes.

The in vivo chemotherapeutic testing indicated that A. catechu, C. pepo, and U. macrocarpa, singly or combined, did not seem to be effective against H. diminuta. The % efficacies effected by A. catechu, C. pepo, and U. macrocarpa were lower than that by niclosamide and arecoline hydrobromide. Arecoline hydrobromide, however, had some adverse side-effects, immediately after treatment but the rats recovered quickly. The low % efficacies obtained with A. catechu as compared to laboratory prepared arecoline hydrobromide, implied that the raw herb contained a minimal amount of the active ingredient. Consequently, the method of extraction of the herb is important. Various herbs with active ingredients could have been ignored because of inefficient methods of extraction.

PART IV



General discussion

The laboratory evaluation indicated that Areca catechu, Cucurbita pepo, Melia azedarach, Myrica latifolia, Quisqualis indica, and Ulmus macrocarpa had either little or no anthelmintic activity. The negative results obtained in a screening of plant materials are often accepted per se. The herbs tested, however, have been prescribed in anthelmintic prescriptions for over two thousand years. The herbal treatments investigated are part of a traditional Chinese medical system which is recognized as a rational (Croizier, 1968; Dubos, 1979; Por-kert, 1976a) as opposed to a magico-religious system. This discrepancy leads to the questioning of certain basic assumptions.

The criterion for evaluation "cure" in the experiments was the % reduction of parasites. The statistical analyses determined whether the traditional Chinese herbal anthelmintics (TCHAs) were significantly different from the controls and the Western drugs. The number of adult Nippostrongylus brasiliensis recovered was counted using a dissecting microscope. If the method of historicism¹ is adopted in the evaluation of the efficacy of TCHAs, this situation might not be so simplistic. Since anthelmintic prescriptions listed general and vague after-effects like "all the worms will come out", or "all the worms will be dissolved", while others stated more specifically that "the disease is cured when the mother worm is passed out" for tapeworms, and that "if the worms are either vomited or passed out before the patient eats any food the disease may be considered cured" for Ascaris lumbricoides, the traditional Chinese doctor had to rely on external signs and the pulse, for symptoms. The expulsion of A. lumbricoides which could accompany the

fasting often recommended before medication, and the destruction of cestodes, could be misconstrued as effected by the TCHAs. Diagnostic prejudice and error could later be misinterpreted as cure. The criteria of "cure" used by the traditional Chinese doctors are less stringent and exacting than those used in my experiments. Consequently, the anthelmintic efficacy of the TCHAs could have been over-rated in previous literature.

The process of "healing" usually involves two related components : the control of disease at the physiological level and the "provision of meaning for the individual's experience of it" (Kleinman and Sung, 1979). When the healing process, which includes the therapeutic approach and outcomes, coincides with the health values of the patient which is comprised of the patient's needs, expectations, and feelings, the therapy is often considered to be successful on a symbolic level even when empirical evidence of efficacy is lacking (Kleinman, 1975; Kleinman, 1978; Kleinman and Sung, 1979). This situation is analagous to the placebo effect. Frank (1974) noted that until the last few decades, most medications were pharmacologically inert, if not actually harmful. This is also reflected in the statement : "the history of medical treatment for the most part, until relatively recently is the history of the placebo effect" (Shapiro, 1959).

If the anthelmintic mode of action of A. catechu, C. pepo, M. azedarach (seed and root), M. lapidescens, Q. indica, and U. macrocarpa, are effected through the host's response rather than against the parasite, the negative experimental results could be attributed to the difference in the physiology of the host. Other factors however, are limiting, if the above does not hold true. The use of the Western

reference drugs, pyrantel pamoate, and niclosamide ensured that

N. brasiliensis and H. diminuta were close to the target human parasites.

Historical documents report the efficacy of TCHAs on human beings. The therapeutic value of TCHAs is the result of a complex interplay of psychological, social, and cultural factors with that of the chemotherapeutic and placebo effects. If the evaluation of the efficacy of TCHAs is based on historical documentation alone, the total effect of the various contributing factors, as represented by an interaction $E_h, S, C, Ps, Ph,$ and T (pp. 63).

The laboratory evaluation on rats measures only the chemotherapeutic effect, which is, one component of the total, E_T . If the efficacy of a TCHA in historical literature and Chinese medical texts coincides with that of a laboratory evaluation, the chemotherapeutic effect contributes greatly to the total effect of the medicine, E_T . If they are specified as ineffective in historical documentations and literature, then $E_h, S, C, Ps,$ and Ph react negatively and antagonistically with the chemotherapeutic effects. Psychological (including the placebo effect), social and cultural factors must exert important influences on the therapeutic effect if a laboratory evaluation does not support a positive historical documentation, as seen in the present study.

The Systems Theory is essential to this study, since the different approaches study the problem from various viewpoints; hence a more holistic picture is obtained. Since politics, culture, and psychological factors seem to play such a major role in the efficacy of TCHAs, it would be interesting to compare the efficacy of TCHAs in a Chinese community, a community which has been previously exposed to

the Chinese medical system but are not ethnically Chinese, and a community which has no previous exposure to the Chinese medical system or does not believe in it. It is also noteworthy that African folk medicine too recommends the leaves from a tree of the family Combretaceae, as an anthelmintic treatment (Ayensu, 1978; Harley, 1970).

Q. indica also belongs to the family Combretaceae. The alcoholic extract from the stem of Amura wallichii (Meliaceae) was found to be anthelmintic against Ascaridia galli (Satyavati et al., 1976). It is also noted in Chinese medical texts that M. azedarach (seed and root), which belongs to Meliaceae, is active against A. lumbricoides. Similar relationships, thus, exist between other herbal remedies in widely separated geographical areas. It would be a remote chance that the dissemination of this knowledge from China to Africa or vice versa occurred some thousand of years ago. The only other possible explanation for this convergence is that active anthelmintic substances do exist in the families Combretaceae and Meliaceae.

From the laboratory evaluation, it can only be concluded that the effect of A. catechu, C. pepo, M. azedarach, M. lapidescens, and U. macrocarpa, were not significantly different from the untreated controls. Only 0.900 g Q. indica/ml was found to be significantly different from the controls but its 31% reduction is very much lower than that obtained with pyrantel pamoate. The anthelmintic effects of pyrantel pamoate and niclosamide were significantly more efficacious than the TCHAs and the controls. The chemical efficacy of TCHAs could hardly be due to its chemical effect alone. The social and psychological factors must contribute greatly to its efficacy.

This study, in addition to reviewing the complex socio-cultural

and historical aspects of traditional Chinese medicine, must conclude that often in animal models, Western drugs are far superior and that the herbal anthelmintics investigated, with the exception of Q. indica, are not chemotherapeutically effective against N. brasiliensis and H. diminuta in rats and A. lumbricoides under in vitro conditions.

Footnotes:

1. This definition of historicism : " an approach which emphasized the uniqueness of all historical phenomena and maintained that each age should be interpreted in terms of its own ideas and principles, or negatively, that the actions of men in the past should not be explained by reference to the beliefs, motives, and valuations of the historian's own epoch ", rather than in the sense used by Popper, that is, " the belief in large-scale laws of historical development of the kind to be found in speculative systems of history, whether linear or cyclic, such as those of Hegel, Marx, Comte, Spengler, and Toynbee " (Bullock, A.O. and O. Stallybrass, eds. 1977. The Harper Dictionary of Modern Thought. pp. 285-286. New York: Harper and Row, Publishers) is used here.

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APPENDICES

Appendix 1 : Conversion Tables.

Weight

1 fen	分	=	0.36 g
1 ch'ien	錢	=	3.60 g
1 liang	兩	=	36.00 g

(Chao, 1940)

Volume

1 cup	碗	=	237 ml
1 sheng	升	=	1000 ml
1 kên	合	=	100 ml

(A Barefoot Doctor's Manual,
1977)Length

1 li	里	=	500 m

(Xinhua Zidian, 新華字典
1971)

Appendix 2 : Dosage calculations (for Experiment 1)

Human dose

<u>M. azedarach</u>	3 ch'ien ¹	=	10.8 g
<u>Q. indica</u>	3 ch'ien	=	10.8 g
<u>M. lapidescens</u>	3 ch'ien	=	10.8 g

The Chinese traditionally prepare their herbal medicine by boiling the herbal ingredients in water in a clay pot until eight-tenths of the solution remained. The final volume was one bowl.

$$\begin{aligned}
 1 \text{ bowl} &= 240 \text{ ml} \\
 \text{Initial volume} &= 240 \times \frac{10}{8} = 300 \text{ ml}
 \end{aligned}$$

The human dose was

$$\frac{10.8 \times 3}{240} = 0.135 \text{ g/ml.}$$

Since the metabolic rate of rats is twelve times that of humans (Prosser, 1973; Taylor, Schmidt-Nielsen, and Raab, 1970), the rat dose should be

$$\frac{10.8 \times 3 \times 12}{240} = 1.62 \text{ g/ml}$$

1. See Appendix 1 : Conversion Tables.

Appendix 3 : Dosage calculations for pyrantel pamoate.

Human dose

$\frac{1}{2}$ teaspoonful per 26 lb (= 12 kg body weight)

10 ml for 60 kg body wt.

$$\begin{aligned}\text{Human dose} &= \frac{10}{60\,000} \text{ ml/(g body wt)} \\ &= 1.67 \times 10^{-4} \text{ ml/(g body wt)}\end{aligned}$$

The metabolic rate of rats is twelve times that of humans
(Prosser, 1973; Taylor, Schmidt-Nielsen, and Raab, 1970).

$$\text{Rat dose} = 1.67 \times 10^{-4} \times 12 = 2.00 \times 10^{-3} \text{ ml/(g wt rat)}$$

A 112 g rat should be administered with

$$\frac{10}{60\,000} \times 12 \times 112 = 0.224 \text{ ml pyrantel pamoate}$$

1 ml Combantrin contains 144 mg pyrantel pamoate

$$= 50 \text{ mg pyrantel base}$$

The rat dose is equal to

$$2.00 \times 10^{-3} \times 144 = 0.288 \text{ mg pyrantel pamoate/(g wt rat)}$$

Appendix 4 : Experiment 1 - The effect of a 1:1:1 mixture of M. azedarach, M. lapidescens, and Q. indica and pyrantel pamoate on the recovery of adult N. brasiliensis in rats.

Replication \ Treatment	Control	1:1:1 mixture of <u>M. azedarach</u> (seed), <u>M. lapidescens</u> , and <u>Q. indica</u>	Pyrantel pamoate
1	96	42	0
2	104	59	0
3	144	81	0
4	148	123	0
5	198	182	0
$\sum X$	690	487	0
$\bar{X}$	138.0	97.4	0.0
% efficacy	0.00	29.42	100.00
Standard error	18.24	25.12	0.00
95% confidence limits	50.72	69.85	0.00

Appendix 5 : Experiment 2 - The weight of rats and the weight of rat chow eaten by uninfected rats.

Day of experiment	Animal No.	Control		Treatment					
		Weight of rat (g)	Weight of rat chow eaten daily (g)	<u>M. azedarach</u> Weight of rat (g)	Weight of rat chow eaten daily (g)	<u>Q. indica</u> Weight of rat (g)	Weight of rat chow eaten daily (g)	<u>Pyrantel pamoate</u> Weight of rat (g)	Weight of rat chow eaten daily (g)
0	1	110.4	15.4	101.2	13.9	101.9	13.6	85.9	15.7
	2	108.2	13.0	109.2	11.7	104.2	17.5	116.2	19.7
1	1	121.4	18.1	112.5	17.5	114.7	8.0	110.0	18.5
	2	118.4	17.6	120.1	15.0	125.6	17.8	126.5	21.3
2	1	131.5	18.6	120.6	16.4	123.0	16.0	118.3	17.0
	2	126.8	13.8	127.8	13.9	133.6	18.8	136.0	18.1
3	1	138.6	18.6	128.6	16.3	130.8	16.4	126.1	15.7
	2	136.2	20.9	136.4	17.9	137.8	16.4	144.0	21.3
4	1	146.2	-	133.8	-	139.7	-	130.8	-
	2	142.5	-	141.9	-	143.3	-	151.3	-
5	1	154.9	23.0	145.6	20.1	147.0	18.2	137.8	16.7
	2	154.1	21.7	154.6	19.1	154.2	20.2	161.8	21.7

(cont.)

Appendix 5 (cont.)

Day of experiment	Animal No.	Control		Treatment					
				<u>M. azedarach</u>		<u>Q. indica</u>		Pyrantel pamoate	
		Weight of rat (g)	Weight of rat chow eaten daily (g)	Weight of rat (g)	Weight of rat chow eaten daily (g)	weight of rat (g)	Weight of rat chow eaten daily (g)	weight of rat (g)	weight of rat chow eaten daily (g)
6	1	161.5	20.7	149.4	20.0	154.0	20.4	143.5	19.4
	2	157.5	24.1	157.9	21.4	160.0	20.9	170.8	23.6
7	1	170.4	19.5	159.3	17.0	163.1	17.9	152.1	15.7
	2	168.3	20.3	164.9	19.4	166.5	19.1	175.7	19.5
8	1	181.9	17.6	164.0	19.2	171.5	18.6	154.8	18.7
	2	178.1	19.0	172.8	15.0	170.6	16.9	182.3	17.9
9	1	184.7	19.5	170.0	18.3	178.0	19.1	162.3	15.6
	2	185.8	22.2	177.0	18.7	171.9	18.5	183.9	20.8

Appendix 6 : Experiment 2 - The weight of rats and the weight of rat chow eaten by infected rats.

Day of experiment	Animal No.	Control		Treatment					
		Weight of rat	Weight of rat chow eaten daily	<u>M. azedarach</u>		<u>Q. indica</u>		Pyrantel pamoate	
		(g)	(g)	Weight of rat (g)	Weight of rat chow eaten daily (g)	Weight of rat (g)	Weight of rat chow eaten daily (g)	Weight of rat (g)	Weight of rat chow eaten daily (g)
0	1	106.4	17.5	115.5	16.7	111.2	16.5	100.7	0.3
	2	108.3	16.0	106.0	15.0	102.5	16.7	92.7	16.1
	3	108.3	11.7	104.0	14.6	106.0	18.0	104.9	14.6
	4	98.9	14.1	108.0	16.4	103.0	15.3	110.0	16.5
1	1	104.7	16.1	119.1	20.4	124.0	19.0	108.9	16.2
	2	117.0	13.7	114.1	15.3	113.0	17.2	100.0	15.0
	3	117.8	16.2	110.4	15.5	118.6	18.5	112.0	13.7
	4	112.8	14.3	117.4	17.7	111.0	16.6	119.5	16.6
2	1	123.9	16.2	131.5	18.2	135.2	19.2	118.5	18.4
	2	123.5	16.6	121.0	18.3	118.5	18.7	106.8	16.0
	3	126.5	17.3	117.3	13.5	127.4	18.1	119.4	15.3
	4	114.9	13.4	125.0	14.4	120.2	17.6	128.7	20.1
3	1	132.4	16.7	138.8	20.1	145.0	19.3	127.4	18.1
	2	129.8	17.2	126.9	14.9	126.9	21.2	112.0	13.7
	3	133.3	17.4	125.4	17.6	137.2	19.3	125.8	16.3
	4	125.8	15.4	134.0	16.1	131.7	14.1	135.2	18.4
4	1	140.5	-	148.5	-	152.1	-	136.5	-
	2	143.4	-	137.1	-	135.7	-	121.3	-
	3	143.0	-	130.0	-	142.0	-	135.4	-
	4	132.0	-	140.5	-	135.7	-	144.9	-

(cont.)

Appendix 6 (cont.)

Day of experiment	Animal No.	Control		Treatment					
		Weight of rat (g)	Weight of rat chow eaten daily (g)	<u>M. azedarach</u>		<u>Q. indica</u>		Pyrantel pamoate	
				Weight of rat (g)	Weight of rat chow eaten daily (g)	Weight of rat (g)	Weight of rat chow eaten daily (g)	weight of rat (g)	weight of rat chow eaten daily (g)
5	1	147.5	16.9	161.5	23.2	162.2	20.0	145.5	20.7
	2	149.9	15.1	144.6	19.9	141.7	18.7	127.7	16.7
	3	153.0	19.5	137.4	19.8	150.0	19.3	145.2	18.7
	4	137.5	15.8	148.3	19.7	146.6	18.9	155.6	20.9
6	1	150.0	21.4	169.2	22.0	173.8	24.0	154.6	19.9
	2	158.6	21.7	154.6	18.1	149.5	19.2	133.9	19.0
	3	161.1	19.1	146.5	19.1	164.5	24.3	153.5	22.3
	4	146.8	16.5	158.0	20.4	155.2	19.4	161.3	22.8
7	1	163.8	20.9	177.3	21.3	175.8	21.5	162.8	21.4
	2	164.6	17.6	161.8	19.5	155.5	18.6	141.6	18.2
	3	165.3	18.7	147.5	13.2	171.9	19.8	162.6	18.5
	4	152.2	15.1	163.5	19.1	162.5	19.7	168.2	26.1
8	1	176.2	19.3	177.8	17.7	186.8	16.9	172.7	18.7
	2	176.5	19.5	172.6	19.1	162.4	18.7	147.8	15.7
	3	173.8	16.9	156.3	16.3	181.8	22.7	168.2	18.7
	4	159.0	15.0	174.3	19.4	170.8	17.9	173.3	16.3
9	1	182.2	20.8	185.0	19.0	191.5	21.5	180.0	22.0
	2	187.6	22.1	180.8	21.5	168.1	19.3	154.1	18.8
	3	181.8	20.3	162.7	17.5	190.0	21.6	175.2	20.2
	4	164.7	17.7	177.8	21.3	174.9	20.4	178.1	19.9

Appendix 7: Experiment 2 - The effect of M. azedarach, Q. indica, and pyrantel pamoate on the recovery of adult N. brasiliensis in rats.

Treatment Replication	Control	<u>M. azedarach</u>	<u>Q. indica</u>	Pyrantel pamoate
1	115	102	74	0
2	131	120	108	0
3	164	121	120	0
4	195	135	148	0
Σx	605	478	450	0
$\bar{x}$	151.3	119.5	112.5	0.0
% efficacy	0.00	20.99	25.62	100.00
Standard error	17.80	6.76	15.33	0.00
95% confidence limits	56.59	21.51	48.74	0.00

Appendix 8 : Experiment 3 - The weight of uninfected rats.

Day of experiment	Animal No.	Control Weight of rat (g)	Treatment		
			<u>Q. indica</u> Weight of rat (g)	<u>M. lapidescens</u> weight of rat (g)	Pyrantel pamoate weight of rat (g)
0	1	62.0	62.8	62.8	63.7
	2	72.7	71.5	71.5	72.2
	3	53.2	56.3	56.8	52.6
1	1	62.6	64.2	61.9	61.2
	2	76.0	74.5	73.8	72.4
	3	58.5	59.0	58.5	56.9
2	1	69.8	67.6	68.2	68.3
	2	81.3	82.5	81.9	77.8
	3	64.4	67.3	64.3	62.0
3	1	78.9	76.6	75.0	76.9
	2	90.5	89.9	90.9	84.9
	3	72.6	74.2	70.1	68.6
4	1	86.0	83.7	78.3	83.8
	2	97.0	97.8	97.6	93.7
	3	77.7	80.7	75.5	74.8
5	1	93.3	89.8	86.5	89.7
	2	105.9	107.0	109.5	102.2
	3	86.2	87.4	82.0	84.4

(cont.)

Appendix 8 (cont.)

Day of experiment	Animal No.	Control Weight of rat (g)	Treatment		
			<u>Q. indica</u> Weight of rat (g)	<u>M. lapidescens</u> Weight of rat (g)	Pyrantel pamoate Weight of rat (g)
6	1	96.1	97.9	93.0	95.2
	2	115.5	110.6	114.1	106.8
	3	92.2	91.6	86.3	91.1
7	1	107.2	106.2	99.0	104.5
	2	126.7	123.5	124.2	114.2
	3	100.7	102.3	96.3	99.8
8	1	103.8	113.0	102.9	107.0
	2	132.8	123.0	130.3	121.4
	3	108.1	106.3	100.3	105.2
9	1	119.7	118.0	112.6	114.4
	2	144.3	129.2	139.0	128.2
	3	113.5	114.0	107.8	111.2

Appendix 9 : Experiment 3 - The weight of infected rats.

Day of experiment	Animal No.	Control Weight of rat (g)	Treatment		
			<u>Q. indica</u> Weight of rat (g)	<u>M. lapidescens</u> Weight of rat (g)	Pyrantel pamoate weight of rat (g)
0	1	55.1	55.5	55.5	55.2
	2	58.9	60.0	60.6	57.1
	3	65.6	66.1	66.4	64.9
	4	69.1	73.3	68.2	69.2
1	1	58.5	55.5	58.6	56.0
	2	62.2	58.6	62.2	58.2
	3	66.4	66.4	64.5	65.2
	4	68.9	72.6	67.7	63.9
2	1	64.3	61.6	64.4	60.3
	2	68.0	66.9	66.8	62.5
	3	73.0	74.2	70.5	73.8
	4	75.4	79.3	72.5	70.5
3	1	71.3	71.0	69.6	66.7
	2	74.9	73.7	74.4	71.5
	3	82.4	82.1	76.9	78.9
	4	81.8	87.3	80.7	79.7
4	1	77.7	76.1	79.6	71.5
	2	86.2	81.2	83.4	76.9
	3	89.7	87.6	80.9	82.3
	4	86.7	92.0	85.4	87.0

(cont.)

Appendix 9 (cont.)

Day of experiment	Animal No.	Control Weight of rat (g)	Treatment		
			<u>Q. indica</u> Weight of rat (g)	<u>M. lapidescens</u> Weight of rat (g)	Pyrantel pamoate weight of rat (g)
5	1	86.2	86.6	86.4	77.3
	2	94.7	89.6	90.7	81.6
	3	99.3	97.3	87.3	86.1
	4	84.1	99.3	92.9	95.0
6	1	91.1	92.0	92.1	83.9
	2	99.5	97.1	97.6	87.8
	3	103.2	101.9	92.0	94.5
	4	90.5	107.4	99.6	101.6
7	1	100.3	100.2	100.0	86.3
	2	110.6	106.9	107.3	94.2
	3	112.9	108.8	101.1	102.1
	4	100.3	119.9	106.0	109.0
8	1	106.6	105.1	104.8	91.2
	2	114.8	117.4	112.3	96.1
	3	119.2	113.2	105.8	108.5
	4	107.8	121.4	112.4	115.6
9	1	112.8	111.2	110.0	96.6
	2	120.8	123.2	119.0	104.5
	3	125.7	120.2	112.9	117.5
	4	111.7	129.6	120.3	123.4

Appendix 10 : Experiment 3 - The effect of Q. indica, M. lapidescens, and pyrantel pamoate on the recovery of adult N. brasiliensis in rats.

Replication \ Treatment	Control	<u>Q. indica</u>	<u>M. lapidescens</u>	Pyrantel pamoate
1	298	358	370	0
2	411	366	453	0
3	429	421	456	1
4	498	498	492	11
$\sum X$	1636	1643	1771	12
$\bar{X}$	409.0	410.8	442.8	3.0
% efficacy	0.00	- 0.43	- 8.25	99.27
Standard error	41.48	32.28	25.82	2.68
95% confidence limits	131.90	102.64	82.10	8.51

Appendix 11 : Experiment 4 - The effect of A. catechu and C. pepo (a), Q. indica (b), M. azedarach (c), (ab), (bc), (ac), and (abc) on the recovery of adult N. brasiliensis in rats.

Treat- ment Repli- cation	Control	<u>A. catechu</u> and <u>C. pepo</u>	<u>Q. indica</u>	<u>M. azedarach</u>	(ab)	(bc)	(ac)	(abc)
1	491	490	465	560	688	527	632	622
2	590	591	551	660	765	626	713	664
3	592	592	557	779	789	725	737	721
$\sum x$	1673	1673	1573	1999	2242	1878	2082	2047
$\bar{x}$	557.7	557.7	524.3	666.3	747.3	626.0	694.0	682.3
% efficacy	0.00	0.00	5.98	- 19.49	- 34.01	- 12.25	- 24.45	- 22.35
Standard error	33.34	33.83	29.72	63.60	30.46	57.16	31.76	19.34
95% confidence limits	143.35	145.49	127.78	272.19	131.00	245.78	136.59	83.17

Appendix 12 : Experiment 5 - The effect of different concentrations of Q. indica on the recovery of adult N. brasiliensis in rats.

Treatment Replication	Control	Concentration of <u>Q. indica</u> (g/ml)		
		0.225	0.450	0.900
1	725	758	615	473
2	770	427	480	310
3	646	489	564	543
4	625	633	562	624
$\sum x$	2766	2307	2221	1950
$\bar{x}$	691.5	576.8	555.3	487.5
% efficacy	0.00	16.59	19.70	29.50
Standard error	31.23	74.24	27.92	66.73
95% confidence limits	107.75	236.09	88.79	212.19

Appendix 13 : Experiment 6 - The effect of different concentrations of Q. indica on the recovery of adult N. brasiliensis in rats.

Treatment Replication	Control	Concentration of <u>Q. indica</u> (g/ml)		
		0.225	0.450	0.900
1	829	787	705	687
2	637	620	299	290
3	534	532	516	463
4	783	608	449	466
Σx	2783	2547	1969	1906
$\bar{x}$	695.8	636.8	492.3	476.5
% efficacy	0.00	8.48	29.25	31.51
Standard error	67.69	53.74	84.18	81.34
95% confidence limits	215.25	170.89	267.70	258.65

Appendix 14 : Experiments 5 and 6 - The effect of different concentrations of Q. indica on the recovery of adult N. brasiliensis in rats.

Treatment Replication	Control	Concentration of <u>Q. indica</u> (g/ml)		
		0.225	0.450	0.900
1	725	758	615	473
2	770	427	480	310
3	646	489	564	543
4	625	633	562	624
5	829	787	705	687
6	637	620	299	290
7	783	608	449	466
8	534	532	516	463
$\sum X$	5549	4854	4190	3856
$\bar{X}$	693.6	606.8	523.8	482.0
% efficacy	0.00	12.52	24.49	30.51
Standard error	35.05	43.77	42.75	48.74
95% confidence limits	82.72	103.64	100.89	115.04

Appendix 15 : Experiment 7 - The effect of A. catechu and C. pepo, U. macrocarpa, and niclosamide on the number of adult H. diminuta recovered from rats.

Treatment Replication	Control	<u>A. catechu</u> and <u>C. pepo</u>	<u>U. macrocarpa</u>	Niclosamide
1	3	3	3	0
2	1	5	1	0
3	1	4	3	0
4	1	2	1	0
5	3	2	2	0
6		3	2	
ΣX	9	19	12	0
$\bar{X}$	1.8	3.2	2.0	0
N	5	6	6	5
% efficacy	0.00	- 75.93	- 11.11	100.00
Standard error	0.49	0.48	0.37	0.00
95% confidence limits	1.36	1.23	0.94	0.00

Appendix 16 : Experiment 7 - The effect of A. catechu and C. pepo, U. macrocarpa, and niclosamide on the dry weight of adult H. diminuta recovered from rats.

Treatment Replication	Control	<u>A. catechu</u> and <u>C. pepo</u>	<u>U. macrocarpa</u>	Niclosamide
1	0.43	0.41	0.43	0.00
2	0.24	0.28	0.28	0.00
3	0.26	0.55	0.39	0.00
4	0.07	0.36	0.19	0.00
5	0.32	0.32	0.13	0.00
6		0.20	0.27	0.00
$\sum X$	1.32	2.12	1.69	0.00
$\bar{X}$	0.22	0.35	0.28	0.00
N	5	6	6	5
% efficacy	0.00	- 33.71	-6.82	100.00
Standard error	0.06	0.05	0.05	0.00
95% confidence limits	2.78	2.57	2.57	2.78

Appendix 17 : The effect of A. catechu and C. pepo (a), U. macrocarpa (b), and (ab) on the number of adult H. diminuta recovered from rats.

Replication \ Treatment	Control	<u>A. catechu</u> and <u>C. pepo</u>	<u>U. macrocarpa</u>	<u>A. catechu, C. pepo</u> and <u>U. macrocarpa</u>
1	3	5	7	5
2	6	4	5	0
3		4	4	8
4		4	4	4
$\sum x$	9	17	20	17
$\bar{x}$	4.5	4.3	5.0	4.3
N	2	4	4	4
% efficacy	0.00	5.56	11.11	5.56
Standard error	1.50	0.25	0.71	1.65
95% confidence limits	19.06	0.80	2.25	5.25

Appendix 18 : Experiment 8 - The effect of A. catechu and C. pepo (a), U. macrocarpa (b), and (ab), on the dry weight of adult H. diminuta recovered from rats.

Treatment Replication	Control	<u>A. catechu</u> and <u>C. pepo</u>	<u>U. macrocarpa</u>	<u>A. catechu, C. pepo,</u> and <u>U. macrocarpa</u>
1	0.30	0.23	0.48	0.56
2	0.50	0.54	0.32	0.00
3		0.31	0.44	0.80
4		0.51	0.53	0.50
$\sum x$	0.80	1.59	1.77	1.86
$\bar{x}$	0.40	0.40	0.44	0.47
N	2	4	4	4
% efficacy	0.00	0.63	- 10.63	- 16.25
Standard error	0.10	0.08	0.04	0.17
95% confidence limits	12.71	3.18	3.18	3.18

Appendix 19 : * Experiment 9 - The effect of different concentrations of A. catechu and arecoline hydrobromide on the number of adult H. diminuta recovered from rats.

Treatment Replication	Control	Concentration of <u>A. catechu</u> (g/ml)			Concentration of arecoline (g/g wt rat)	
		1.6	3.2	6.4	0.00061	0.00123
1	5	9	8	7	0	6
2	9	7	6	6	0	0
3	8		9	4	0	0
4				8		0
Σx	22	16	23	25	0	6
$\bar{x}$	7.33	8.00	7.67	6.25	0.00	1.50
N	3	2	3	4	3	4
% efficacy	0.00	9.09	4.55	14.77	100.00	79.55
Standard error	1.20	1.00	0.88	0.85	0.00	1.50
95% confidence limits	5.17	12.71	3.79	2.72	0.00	4.77

Appendix 20 : Experiment 9 - The effect of different concentrations of A. catechu and arecoline hydrobromide on the dry weight of adult H. diminuta recovered from rats.

Treatment Replication	Control	Concentration of <u>A. catechu</u> (g/ml)			Concentration of arecoline (g/g wt rat)	
		1.6	3.2	6.4	0.00061	0.00123
1	0.26	0.75	0.38	0.45	0.00	0.54
2	0.47	0.66	0.56	0.42	0.00	0.00
3	0.42		0.53	0.23	0.00	0.00
4				0.58		0.00
$\sum X$	1.15	1.41	1.47	1.68	0.00	0.54
$\bar{X}$	0.38	0.71	0.49	0.42	0.00	0.14
N	3	2	3	4	3	4
% efficacy	0.00	- 83.91	- 27.83	- 9.57	100.00	63.78
Standard error	0.06	0.04	0.06	0.07	0.00	0.14
95% confidence limits	4.30	12.71	4.30	3.18	4.30	3.18

Appendix 21 : Preparation of concentrated artificial perienteric fluid (APF) for A. lumbricoides.

Before using, the concentrated resulting solutions were diluted to 30% (v/v) with distilled water.

Compounds	Identification of chemicals	Weight (g)
CH ₃ COONa anhydrous F.W. 82.03	Anachemia Lot 471128	34.22
NaCl F.W. 58.44	Anachemia	0.75
KCl F.W. 74.56	Fisher Scientific Comp. Lot 742105	6.10
MgCl ₂ · 6H ₂ O F.W. 203.33	Anachemia Lot 760308	3.29
CaCl ₂ anhydrous M.W. 110.99	Anachemia Lot 860927	2.19
Sucrose C ₁₂ H ₂₂ O ₁₁ M.W. 342.30	Aristar BDH Chemicals Ltd. Lot 1786080	8.60

From del Castillo, J., and T. Morales. 1969. Electrophysiological experiments in Ascaris lumbricoides. In: Experiments in Physiology and Biochemistry, ed. G.A. Kerkut, pp. 222. London: Academic Press.

Appendix 22 : The operation of Grass Model 7 Polygraph.

1. Connect the transducer to the input of the 5 P 1 or 7 P 1 Polygraph Preamplifier.
2. Set the input selector switch to the "BRIDGE 2K" position.
3. Establish the base line on the Driver Amplifier as outlined in the Driver Amplifier Instruction Manual.
4. Set the Driver Amplifier POLARITY-CAL-USE switch to USE.
5. Set the sensitivity controls on the 5 P 1 or 7 P 1 to a low value.
6. Return the pen to the base line (established in 3) with the Balance Voltage controls.
7. Increase the sensitivity and readjust the Balance Voltage control to set the pen to the established base line.
8. Reduce the sensitivity to a value suitable to the calibration weight or force used. Using the fine sensitivity control set up a decimal scale relation between grams of force on the transducer and mm deflection of the pen. Once this relationship has been established, it is possible to set the Sensitivity step control to other convenient decimal equivalent scales.
9. If desired, the Balance Voltage controls can be used to balance the electrical output caused by static forces on the gauge so that greater sensitivity can be used to measure smaller changes in force. Do not use the Base Line for this purpose.

(Grass Medical Instruments Instruction Manual, 1968)

Appendix 23 : Chemical extraction procedures.

General principles

1. The herbs were finely ground to facilitate extraction.
2. Hexane was added to the ground herbs to extract fat, fatty acids, glyceroids, glycerols, and other less polar substances.
3. Ethanol was added to the fat-extracted residues to extract phenolics, alkaloids, quinones, and other more polar substances.
4. Water was added to resultant residuals to extract amino-acids, proteins, and very polar substances.

The application of the above principles to the extraction of organic compounds from M. azedarach, Q. indica, and M. lapidescens:-

	<u>M. azedarach</u>	<u>Q. indica</u>	<u>M. lapidescens</u>
1. weight of ground herbs	34.6 g	35.2 g	20.3 g
Time of grinding	45 s	30 s	30 s

2. Volume of hexane used	175 ml	175 ml	100 ml

The flasks were placed above a warm bath (30°C), occasionally stirring, for 30 mins.

Temperature of extract after removal from the warm bath.	37°C	41°C	45°C

3. The hexane of the hexane extract was evaporated using a Buchi rota-vapourer until a constant weight was obtained. The temperature of the water was about 40°C; the temperature of the solvent was about 35°C.

Appendix 23 (cont.)

	<u>M. azedarach</u>	<u>Q. indica</u>	<u>M. lapidescens</u>

4. The fat-extracted materials were dried at 40°C to evaporate off any remaining hexane.

Weight of fat-soluble extract	0.5 g	1.1 g	100 mg

5. Methanol extracted "smaller" molecules which could dissolve in methanol. Cross-linked and other larger molecules were not soluble in methanol, and they could be extracted with ethanol after the methanol extraction.

Methanol extraction possible.	Methanol insufficient extraction to do a methanol extraction.

Volume of ethanol used	175 ml	175 ml	100 ml

The solutions were left in a warm bath at about 40°C for 30 minutes.

Fluorescence in the ethanol extracts indicated the presence anthraquinones.

Slight fluorescence observed.	Fluorescence observed.	Fluorescence not observed.

6. The residuals, insoluble in hexane or ethanol/methanol were dried at 90°C for 90 mins.
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