

Sir Aldo Castellani Memorial Lecture - 1993

## Aldo Castellani — a paradigm for a medical career

S N Arseculeratne<sup>1</sup>

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Madame President and the Ceylon College of Physicians, I feel honoured to have been invited by you to deliver this Sir Aldo Castellani Memorial Lecture and I thank you for your gesture.

At the inaugural Castellani lecture last year, Vitarana reviewed the life and work of this creative man with particular reference to his work in Ceylon. It remains for me to include in this lecture, some aspects of our own work, related to his. His rich biography also allows of some gleanings on more general issues of science, science administration and the world of medicine. In discussing these, I am impressed by the variety of his interests and by the eclecticism of his views and ideas as documented in his fascinating autobiography — 'Microbes, Men and Monarchs'.

It is relevant to remind ourselves that Castellani was born and had his education in Florence which is considered by some to have been the centre of the European Renaissance, that tremendous blooming of literary, artistic, scientific creativity, and of humanism, a flavour of which one cannot fail to discern in Castellani's work and writings. His warmth, sensitivity, liberalism, tolerance, and ebullience so typical of the Italian temperament, are also evident in the narratives on his experiences in foreign lands. He appears to have had a deep awareness of the history of medicine, including the contributions of non-European civilisations in contrast to the common Eurocentric views of most western historians of medicine.

If the challenge of and the response to the new was the essence of the Renaissance, then the choice for Castellani as a medical man, of tropical medicine for further studies, was a natural one. Castellani responded to the challenge posed by obscure tropical diseases, with a series of fundamental discoveries notably in disease-ridden Africa, trypanosomiasis, yaws, and a host of other parasitic, bacterial and fungal diseases. Professor Ito documented an impressive list of Castellani's researches on bacteriology, mycology, parasitology with descriptions of new micro-organisms and new diseases. Speciation is a difficult task, and without the DNA analysis and computer models of today he had to rely on a sharp sense of discrimination in identifying 'new' organisms. The validity, many decades later, of some of Castellani's 'new' species is a tribute to his scientific insights.

For his work on the pathogenesis of disease, Castellani had none of the scientific technology, the sophisticated and versatile equipment nor even the extensive theoretical framework of microbiology and immunology that researchers have today. Yet what he had for his great productivity, was devotion.

Despite our fragmentary treatment of disease in terms of narrow specialities, an understanding of the disease process, from its aetiology, through pathogenesis to management can come only through a syncretic view of the entire disease. That statement is trite but true and yet so often ignored in local research. Castellani's success was based on that broad approach.

In 1904, Castellani published his work on diphtheria in Ceylon. By the 1950s much was known about its bacteriology, although the aetiology of the circulatory failure that sometimes accompanies diphtheria was then controversial. This was my first research problem when I took up a career in microbiology in 1957. Two workers from London reported that the erythema induced by intradermal diphtheria toxin showed a feebler blanching response to locally injected pressor amines and suggested that the circulatory failure of diphtheria was due to a failure of the pressor responses to endogenous vasoconstrictors. It seemed to me that this failure might rather be a general, protective phenomenon in acute inflammation and this was proved on skin inflammation with erythema induced by turpentine and by *Staph. aureus*. It was also suggested that this reduction in the noradrenaline sensitivity of blood vessels in acutely inflamed tissue was due to the products of inflammation. Indeed as Walter & Israel now write in their book on general pathology, "When once inflammation is established the blood vessels fail to react to sympathetic stimulation or a vasoactive drug like adrenaline".

This work was followed by the measurement of pressor responses of directly visualised mesenteric and skin arterioles, to intravenous noradrenaline, to isolate the systemic toxic action from local inflammatory effects, in experimental diphtheritic infection. Zintel chambers which I modified for quantitative observations on the intestine, were made from bits and pieces in our laboratory. It was demonstrated<sup>2</sup> that these vessels indeed showed decreased vasoconstrictor responses. The probable mechanism was suggested by a parallel examina-

tion of the histology of the adrenal cortex, which in these animals showed degeneration and necrosis of the Zona Fasciculata. This zone produces the glucocorticoids which exert a supportive action on the blood vessels in their homeostatic responses to pressor amines in states of stress. Moreover the pattern of depression and response was characteristic of that which follows adrenal insufficiency as reported in other experimental work. "Current Therapy" still advocates large doses of cortico steroids in diphtheritic shock while their use in other forms of infective shock is controversial.

It was thus proposed that the events in diphtheritic circulatory failure began with toxic myocarditis which led to a fall in cardiac output; the adrenal response to this stress in an attempt to maintain tissue perfusion was not sustained by the failing myocardium and the resulting relative ischaemia of the adrenal cortex led to its degeneration and necrosis. In contrast to the decreased local pressor responses following intradermal injection of turpentine, systemically injected turpentine was shown to enhance the responses of mesenteric and skin vessels to noradrenaline while the Zona Fasciculata in these animals showed an increase of cytoplasmic without the degenerative changes seen in diphtheria suggesting that the glucocorticoid response to the stress of turpentine could have caused that enhancement. The responses of isolated diphtheritic blood vessels in vitro to pressor amines might have shown whether the lesion was such a functional one due to the lack of the supportive role of rather than a structural one induced by the diphtheria toxin. However, this work was not pursued when I proceeded abroad on study leave.

Another of Castellani's contributions was anti-typhoid immunisation. Almroth Wright first used an anti-typhoid vaccine prepared from *S.typhi* over a hundred years ago. The need for wider protection against diverse infections prevalent in Castellani's time in the tropical countries in which he worked and the advantages of avoiding multiple injections encouraged him to experiment with multivalent vaccines.

So Castellani turned from his earlier experimental work with multiple bacterial vaccines on rabbits in 1909 in Bonn, Germany, to typhoid and paratyphoid in humans in Ceylon. His work in Ceylon on specific prophylaxis in typhoid is a landmark, for it is the first introduction of combined vaccines. Castellani does not describe his techniques for demonstrating that "...the amount of immunity obtained for each infection was identical with and sometimes higher than that induced by the respective monovaccine in control animals", but he claimed that he was able to demonstrate this in human beings in Ceylon, too.

It is common knowledge that heat killed parenteral

typhoid vaccine induces an antibody response to its constituents; it provides some immunity ranging from 51-81% in endemic areas.

Improvements in water supplies, sanitation and hygiene which should be the primary target in the prevention of typhoid, still remain a dream in poor, tropical countries. Thus new vaccines are continuing to be introduced, and *The Lancet* in August 1992, had a leading article on the options on typhoid vaccines.

Despite the long history of studies on typhoid since the discovery of *Salmonella typhi* in 1880, we are only now beginning to understand the immunology of typhoid, facilitated by the tremendous explosion in immunological knowledge over the last two decades.

The intestinal route of entry of *S.typhi* naturally provokes the development of intestinal immunity in the gut lumen, mediated by secretory anti-typhoid IgA. The bacteraemic phases promote the well marked antibody responses, mainly of IgM, which is conveniently demonstrated by the agglutination reaction in the Widal test. Systemically invasive salmonellae are known to have an intra-cellular habitat and as with mycobacteria, brucellae, and viruses, this leads to the development of cell-mediated immunity. Indeed it is now considered that while circulating antibody, as that induced by vaccination with the parenteral heat killed vaccine, could be protective in the primary bacteraemic stage, it is cell-mediated immunity which determines the recovery from infection with the intracellularly located organism. Thevanesam, then a post-graduate research student in our department in the mid 1970s, re-examined the immunological status in typhoid and demonstrated<sup>3</sup> a well-marked cell-mediated immune response as shown by the leucocyte migration inhibition test for lymphokines from specifically sensitised T-cells, in normal persons, conservancy labourers occupationally exposed to *S.typhi* and in typhoid patients. Parenteral TAB vaccine in perviously unvaccinated and non-diseased persons, was also found<sup>4</sup> to induce a significant cell-mediated T cell response confirming the work of Scandinavian workers but refuting that of Indian workers; the intradermal route gave higher (though statistically non-significant) sensitisation than through the subcutaneous route. It is likely that this killed TAB vaccine merely boosted a basal CMI derived from subclinical exposure to viable *S. typhi* rather than having induced a primary cell-mediated sensitisation. Adjuvant action through skin lipids, skin-resident corynebacteria and the high proportion of dermal Langerhans cells (the dendritic macrophages) would have been additional promoters of the CMI response. Other interesting findings too emerged from this study. The cell mediated immune response but not the humoral response decreased with increasing age of the vaccine suggesting liability of the CMI inducing

antigen(s). Could this be reflected in the incomplete protection afforded by TAB and the greater potency of the live attenuated Ty21a or even the acetone killed and preserved vaccine which as with the Vi component, would preserve the CMI inducing component? Another finding was the development of post vaccination CMI anergy, confirming the work of Indian researchers. Could this anergy contribute to post-vaccination 'provocation typhoid' It was also found that the intradermal route was less liable to induce the anergy, providing an additional justification for the intradermal rather than the subcutaneous route for this heat killed vaccine.

It was later shown by Peiris and his colleagues<sup>5</sup> in our department, that one of the dreaded complications of typhoid, ileal perforation, was associated with B and T cell hyporeactivity in the mesenteric lymph nodes and significantly lower anti-*S. typhi* O and H antibody concentrations than in patients with uncomplicated typhoid. There was no general failure of immune responsiveness as measured by isohaemagglutinin titres. The mechanism of failure of specific anti-*S. typhi* immunity awaits elucidation although it is possible that genetically controlled hyporeactivity to salmonella antigens could be involved as in the non-response of some strains of mice to *S. typhimurium*.

Modifications of whole cell vaccines to preserve antigenicity or immunogenicity have been field-tested elsewhere; these include the acetone killed, freeze-dried vaccine which gave good protection but was expensive to prepare, and the alcoholised vaccine which preserved the Vi antigen of *S. typhi* but fared poorly in field trials. A recent contender is a parenterally administered purified Vi antigen vaccine which gave 60-72% protection in endemic areas.

It is of interest to recall Castellani's views on typhoid vaccine as documented by Parish (1965)<sup>6</sup> on the history of immunisation. "...Inoculation of a vaccine containing some living typhoid organisms (heated one hour at 50°C) gives a higher degree of immunity than the use of dead organisms. Antityphoid vaccination is well conducted by inoculation of 0.5 cc of the usual dead vaccine (Wright) and one week after 1 cc of live vaccine. There is no danger, and both vaccinations may be with live cultures." Although long predating the knowledge of cell mediated immunity and infection by viable *S. typhi*, Castellani seems to have had the acumen that forestalled the use of live cultures for the induction of CMI. Because recently attention has been given to live, attenuated, orally administered vaccines for these could provide adequate sensitisation for the development of cell-mediated immunity while killed vaccines do not do so. Ty21a, a biochemically deficient mutant has thus been used as an oral experimental vaccine in Egypt with 43-96% protection in endemic

areas and 87% protection in previously non-exposed subjects.

Yet, even a hundred years after typhoid vaccine was first introduced, much remains to be done and despite new vaccines the old parenteral killed, whole bacillary vaccine still remains an option.

Castellani's researches on skin diseases reflected his appointment as Lecturer on Dermatology in the Colombo Medical College. In the 1970s one such disease, scabies, interested us. In 1969/70 4500 and in 1976 1990 cases of scabies (/100,000) were recorded in the state hospitals. Our interest was on account of the documented association between scabies, a supervening streptococcal impetigo and the post streptococcal sequel of acute glomerulo-nephritis (AGN) of which 760 cases/100,000 in 1969/70 and 720/100,000 occurred in 1976. Except for data from Trinidad, no description of post-streptococcal AGN in a tropical country was available at that time.

We made a retrospective study<sup>7</sup> of 86 cases of AGN with skin infection and a prospective follow up of 55 with impetigenised scabies. This incidentally was an undergraduate research project on which Charavanapavan then a 3rd year student, worked; such projects have been in progress in our Faculty for the last twenty years.

The age distribution showed that AGN was predominantly a paediatric problem while scabies was the commonest antecedent impetigenised skin lesion.

The area of affected skin bore no relation to the occurrence of AGN or its severity. The modal latent period was 4 weeks with a mean of 9.3.

Noteworthy features were the broad similarity between the streptococcal types in both AGN and impetigenised scabies in this series, and secondly the similarity with the types reported from cases of AGN in other countries, both tropical and temperate. Only one of our strains was typable by the M antigen; this strain had the well recognised M55 antigen of nephritogenic strains.

Group A is the classical precedent of AGN as we too found, while Group C also thought to be causative was found in a few cases.

While 34% of AGN patients and 35% of impetigenised scabetic patients had beta-haemolytic streptococci in the skin, only 8% of the latter group developed urinary abnormalities suggestive of AGN, on follow-up. Several reasons could be adduced for this disparity: (1) the treatment of the pyoderma preventing the development of AGN, (2) the presence of non-nephritogenic strains, (3) genetic or unknown host susceptibility factors (4) the transmission of the nephritogenic strains amongst subjects in close contact. We had no epidemics of AGN as reported in warmer regions of the US and in Trinidad. Our sporadic cases occurred in unrelated families.

Our anti-streptolysin 'O' titres had the pattern reported by workers elsewhere. As in other reports, our ASO titres in AGN were lower than in Acute Rheumatic Fever.

We found a wide variation in nine properties of *Staphylococcus aureus* isolated from the AGN patients suggesting the absence of 'nephritogenic types', if indeed this species is causative of AGN as suggested by one report in 1961. Staphylococci however have been reported to cause immune complex 'shunt nephritis' which supervenes on intravascular infections.

I find references to 'bacterial pseudomycosis' and 'pseudomycetoma' in Professor Kasuke Ito's draft of his undelivered inaugural Castellani lecture on Castellani's descriptions of new diseases and discovery of their aetiology. In my inability to locate Castellani's publications, I am left to assume that he was referring to certain bacterial diseases of both humans and animals which in their pathology closely resemble the entity now called maduromycosis or mycetoma which are caused by a variety of filamentous fungi, and are characterised by chronic, granulomatous lesions with characteristic histological appearances, most striking of which is the presence of a corona of club-like structures around the microbial mass from which arose the name 'ray-fungus'. The classical bacterium which causes this type of lesion, is of course *Actinomyces israelii*, although there are others; *Staphylococcus aureus* which causes botryomycosis, *Pseudomonas* sp and *Actinobacillus lignieresii*. In the 1960s, I encountered a caseo-purulent disease of the large joints of rabbits which also showed actinophytosis and from which was isolated an unusual bacterium, which, not conforming to hitherto described species, was named *Actinobacillus capsulatus*. In the 1980s, the international committee on bacterial taxonomy re-classified, re-named or deleted non-valid species but retained this organism as a valid species of the genus *Actinobacillus*. While it satisfied the third postulate of Koch in reproducing the disease in normal rabbits, a study of the experimental lesions led to a hypothesis on the pathogenesis of the 'ray-fungus' or actinophytotic formation. The mass of capsulated bacilli in the early stages was followed by the peripheral re-distribution of the capsular, probably polysaccharide and insoluble, material around the bacillary mass. It was hypothesised<sup>9</sup> that, with the formation of anti-capsular antibody in the diseased rabbit, the insoluble antigen-capsular antibody complexes remained localised to the periphery of the bacterial mass. With fragmentation of this protein containing complex in a pinnate manner and the osteoclast-like erosion of the complex by phagocytes, this complex breaks up into the characteristic club-shaped projections. I did not pursue this topic to investigate this idea because the lyophilised original strain proved non-viable but I note that a recent text-book has referred to these clubs in actinophytosis as representing antigen-antibody complexes.

Diseases caused by fungi, those much neglected pathogens, also held Castellani's attention. He described several new pathogenic fungi and associated these with new mycoses, notably bronchomycosis and bronchomycetoma. Fungi, tardy in growth in laboratory culture, seldom spectacular in the diseases they cause are yet remarkable in the diversity in their pathological effects. Mycoses however are now of increasing clinical significance on account of the use of therapies, antibacterial or immunosuppressive, among other agents or factors which compromise patients to infection by opportunistic fungi.

Our interest in mycology was first on an infective disease-mycotic keratitis. Corneal ulceration is a very common ophthalmological disease in this country. It can also be a cause of permanent blindness, while much of it is also preventable. Mycotic infection of traumatised corneas has also been well documented in temperate (USA) and tropical countries (India, Nigeria). In 1977/8 and in 1980/1, we studied 67 cases of keratitis. Incidentally this study too was an undergraduate student research project with Sirosha Gunawardena (now genito-urinary surgeon at Galle) and K.P. Ranasinghe. Scrapings from the affected corneas taken by a surgeon were studied microscopically and culturally for fungi and bacteria. Fungal isolates were speciated by Dr Libero Ajello of the CDC in Atlanta, Georgia, USA, and were tested for sensitivity to anti-fungal drugs in our laboratory. Mycotic keratitis was diagnosed in 32% of cases. The pattern of isolates was similar to that reported from both tropical and temperate countries although that of *Paecilomyces farinosus* appears to be a first isolation from keratomycosis. Of bacteria, *Streptococcus pneumoniae* was the commonest as reported in other studies. The anti-fungal drug sensitivity pattern showed, in decreasing order of effectiveness, flucytosine, Nystatin, Amphotericin B, and Econazole. *Fusarium oxysporum* was resistant to all the drugs tested. Our findings emphasised the predominance of this disease in agricultural, domestic and manual workers through trauma from plant materials and soil or other hard, dust or soil contaminated particles. Another important feature was the history of aggravation of the corneal ulceration by topical traditional remedies. A further point is the vital need to exclude a mycotic aetiology before antibacterial or steroidal topical applications are made for these will aggravate the keratitis which could lead to mycotic endophthalmitis, permanent blindness or evisceration of the eye.

Our work was also concerned with toxic diseases from fungi. It was about three years after their discovery in Britain, when we started work on the aflatoxins, those fascinating toxins produced by *Aspergillus flavus* and related species and which include the most potent hepatocarcinogen in experimental rats yet discovered. The sometimes extensive mould spoilage of copra kernels in

our rural areas impressed me. Yet there was nothing in the world literature on aflatoxin contamination of coconut and its derivatives, oil, poonac and desiccated coconut. These were important export products from Ceylon and our export market faced a threat when the United States rejected consignments from the Philippines on account of aflatoxin contamination of their copra. Our Coconut Board, headed by a scientist, was very responsive in supporting our work and recruited a research student Upali Samarajeewa who is now Professor in the Department of Food Science in our University. The studies<sup>9</sup> revealed the extent and origins of contamination, and suggested methods for control. New avenues were opened in more basic aspects, better methods for assay of coconut products including a new method for bioassay using tadpoles<sup>10</sup>. This tadpole method had advantages over the existing ones using guinea pigs or brine shrimp, the most important being the development of nuclear abnormalities of the hepatocytes of the intoxicated tadpoles which is most probably due to the now well characterised primary action of aflatoxin in binding to nuclear DNA. We also developed a new method for production large amounts of these commercially expensive toxins, and gave a first description<sup>12</sup> of intoxication in goats and the mode of intoxication, a method for prevention of toxin accumulation<sup>13</sup>, a new method<sup>14</sup>, subsequently patented, for decontamination of oil and a new phenomenon and perspective on the physiology of toxin production by the fungus. The oil decontamination process resulted from a collaborative effort with our engineering faculty and is a cheap, simple method using sunlight and based on the rapid degradation of aflatoxin by ultraviolet light.

Our field studies investigated a possible aetiological role of aflatoxin in chronic liver disease including cirrhosis of which Rajasuriya and his colleagues in 1970 described one third as having been of cryptogenic origin; they suggested that hepatotoxic factors be investigated. Our work however showed that aflatoxins in Ceylon, in contrast to the situation in some south and southeast Asian countries did not, then, pose a food borne toxic hazard. However we warned against such a hazard arising in the future from some new industrial developments.

Our research on aflatoxin as a cause of hepatic injury led us to a wider project on natural hepatotoxins and chronic liver disease (CLD). This investigation of the sources of toxic liver injury formed a part of a WHO sponsored southeast Asian inter-country project on CLD. We turned to another group of natural hepatotoxins, well documented abroad, which are also hepatocarcinogenic — the pyrrolizidine alkaloids. These are well known to occur in many plants, some of which are used as traditional herbal medicines. These alkaloids can cross the placenta, be absorbed through the skin and are progressively hepatotoxic following even a single dose. Their experimental toxicity is heightened in younger animals,

and by protein malnutrition. Accidental PA poisoning in epidemic proportions in rural India, and 'bush tea disease' with hepatic veno-occlusion and portal hypertension in Jamaica following prolonged consumption of bush teas from *Crotolaria* and *Heliotropium* species are well documented. The implications of these facts in countries such as ours, is obvious.

With our chemical colleagues, we studied 175 medicinal plants of the traditional ayurvedic pharmacopoeia phytochemically and toxicologically. Four of them showed<sup>15,16</sup> the presence of pyrrolizidine alkaloids — *Crotolaria juncea*, *Crotolaria verrucosa*, *Holarrhena antidysenterica* and *Cassia auriculata*. This was the first report of *Cassia* (*Ranawara*) as a pyrrolizidine alkaloid containing genus, and of *Holarrhena antidysenterica* as a species with these alkaloids. It is noteworthy that infusions from flowers and leaves of *Cassia* are widely consumed in this country as herbal 'tea'. Recently our forensic and clinical colleagues described four cases of fatal intoxication following meals which contained leaves of *Cassia bicapsularis* and the histopathology of the liver was compatible with that of pyrrolizidine alkaloid poisoning. Further tests in identifying these alkaloids in the intoxicated liver will be needed in confirmation of the aetiology. However, we regard plant hepatotoxins as providing a greater hazard than aflatoxins for chronic liver disease in this country.

If Castellani yielded to the fascination of the syncretic study of disease whatever its cause, fungi, bacteria, parasites, climate and even psychological trauma, we found that same fascination in non-microbial causes of chronic liver disease. Let me now relate briefly the discovery of the toxic properties of the young shoot of the palmyrah palm *Borassus flabellifer* L.

Having devised a new method for the bioassay of aflatoxins on tadpoles, we studied the specificity of the nuclear abnormalities which intoxicated tadpole liver nuclei showed. This study necessitated the inclusion of a wide variety of natural poisons. The hepatocarcinogenicity of cycasin from the cycad (madu) seed was well known at this time. It appeared that, at least superficially, the boiled-sundried shoot of the palmyrah palm (Kottakilangu or Odiyal) bore some resemblance in taste and appearance to the cycad seed. In retrospect, I do not think, using this flour was a subconscious flirtation with teleology, that toxicity might confer upon it some value in the palm's survival. In feeding experiments on rats, that popular model for experimental hepatotoxicity, 100% flour from the shoot, proved lethal within a week. The acutely intoxicated liver showed marked hepatocellular histopathology<sup>17</sup> and biochemical evidence of mitochondrial dysfunction in particular, confirmed by ultrastructural studies. The hepatotoxicity was transmissible through milk to suckling rats in which the liver and pulmonary lesions were more marked<sup>18</sup>. In addition to ataxia in the rats, their brain

showed neuronal degeneration, necrosis and neuronophagia. A low molecular weight water-soluble neurotoxin was later partially purified in the Medical Research Council's Toxicology Laboratories in Surrey, England<sup>19</sup>. Collaborative research in Mahidol University, Thailand showed clastogenic effects on human lymphocyte nuclei — chromatid gaps and breaks, acentric and dicentric chromosomes<sup>20</sup>. Significant enhancement of sister chromatid exchange, a more sensitive indicator DNA damage was also demonstrated<sup>21</sup> on human lymphocyte nuclei. The National Food Institute, Copenhagen, Denmark showed that flour extracts were mutagenic on the popular Ames' (reversed mutation) test on bacteria<sup>22</sup>. In long term feeding trials, fibrous venous occlusion in both centrilobular hepatic and portal veins was seen<sup>23</sup> recalling the portal venous occlusion in pyrrolizidine alkaloid poisoning leading to portal hypertension. Hepatic fibrosis occurred without regenerative nodules characteristic of cirrhosis. Further work with rat feeding in our laboratory which investigated a possible hepatocarcinogenicity showed instead, the development of rat mixed-cell malignant lymphomas — both undifferentiated and in various stages of differentiation — rather than primary liver cancers<sup>24</sup>. The lymphomas were mainly intra-abdominal associated with the ileal mesentery with lymphomas in other sites as well, the thymic, pulmonary, cervical and axillary regions, some infiltrating into adjacent organs or tissues. Several of these tumours showed secondary infection with low grade pathogens. Hind limb paralysis was seen in a few lymphomas bearing rats. Feeding of the flour to pregnant females resulted in the development of lymphomas in a few of the progeny.

From such a bewildering array of toxic effects, we next chose the lymphomagenicity to concentrate upon, for it confronted us with an interesting aspect of tumour immunology — the origin of lymphoreticular tumours in organ transplant patients under therapeutic immunosuppression. Apart from the clastogenicity and mutagenicity which had already been suggested as contributory, we hypothesised in 1977 that the experimental rat lymphomas arose from a state of immunosuppression or immune dysregulation induced by the palmyrah flour. From collaborative work with immunologists in Thailand in acute and chronic feeding experiments on rats, we confirmed the development of humoral and cell mediated immune suppression<sup>25</sup>. Through further collaborative work in the Department of Medical Microbiology in the University of Malaya, we confirmed these findings in a mouse model<sup>26</sup> and also showed that the suppression was transferable to normal mice by splenic cells from intoxicated mice. Using monoclonal antibodies to T cell markers we also demonstrated that the suppression was mediated by a T suppressor cell generative or sparing effect. In a recent review on the origin of non-Hodgkin lymphomas, Penn (1991) in the US commented — "Possible causes of the

NHLs include oncogenic viruses, disturbed immune surveillance, chronic antigenic stimulation, impaired immunoregulation, carcinogenic effects of immunosuppressive, cytotoxic or other drugs, and genetic susceptibility to lymphomagenesis". In 1983 we discussed the lymphomagenic effect of this flour through all these properties except the role of genetic factors in the rats. We later had some suggestive but unconfirmed and therefore unpublished results on the inhibition of rejection of allogeneic skin and tumour transplants. I have discussed<sup>27</sup> some possible implications of palmyrah consumption on human disease notably in coastal Lanka and southern India.

What is exciting about these findings in relation to palmyrah flour as a possible source of a therapeutic immunosuppressant for transplantation is that it is either an inducer of T suppressors or enhances suppressor functions without lymphoid cell cytotoxicity which immunosuppressive antiproliferative agents in current use, show. Moreover, we found<sup>28</sup> that the neurotoxin prepared by the British MRC Toxicology Unit was not immunosuppressive while the immunosuppressive fraction which our chemical colleagues prepared was not hepatotoxic. It is of great interest that palmyrah-induced immunosuppression was recently confirmed by Swiss workers who are now characterising this effect and attempting to isolate and identify the suppressive factor(s).

At this stage, you might justifiably ask me 'well, what has plant toxicology and tumour immunology got to do with microbiology which you profess?'. In answering that, I have humble recourse to the eclecticism and fascination for science that Castellani so admirably displayed in his work. In Ceylon where he worked from 1903 till 1915 he was Professor of Tropical Medicine, lecturer in Dermatology, Physician in the General Hospital, Director of the Colombo Clinic for Tropical Diseases and then Director of the Bacteriological Institute, now the MRI. I will plead my own defence in the concluding section of this lecture.

We were next confronted with a complex surgical problem which the surgeons in Kandy in the mid 1970s brought to our attention. This afflicted a wide range of humans, from previously, apparently normal children and young adults, to patients with a preceding illness which was either a hypotensive state following vascular event, traumatic shock, or rarely an infective condition. But they all ended up with a patchy, sometimes segmental oedema, congestion or haemorrhage or incipient gangrene of the wall of the jejunum or ileum large lengths of these regions. Milder cases which presented with an acute abdomen, recovered on conservative treatment while others appeared very toxic and died within two days of admission. Some patients needed resection of the affected intestine. The aetiology then was quite obscure, although this syndrome appeared to conform to that described abroad variously as Enteritis Necroticans, Ne-

crotising Enteritis (NE), Necrotising Enterocolitis and Neonatal Necrotising Enterocolitis. The literature on all these was voluminous, with multiple postulates of aetiology, and listing of numerous predisposing or associated factors.

Our study began with histological examinations of the resected intestines and it soon appeared that approximately half of our cases presented novel features<sup>29</sup>. These consisted of an assortment of immunologically mediated reactions suggestive of hypersensitivity. In many cases the reactions were mixed, with Type I and Type III as is often the case with human hypersensitivity. The Type III lesions resembled the antigen-excess Serum Sickness type with extraintestinal lesions as well. We hypothesised from animal models and other evidence that the source of antigens was probably intestinal *Ascaris*. The leucocytoclastic vasculitis which we saw in these cases of NE was absent in the NE which supervened on shock following trauma, vascular accidents such as aneurysmal rupture, in cardiovascular failure and in prematurity with neonatal necrotising enterocolitis. The factor initiating the ischaemia in the latter cases was presumably their low-flow state with micro-vascular shunting away from the intestine.

We then turned to the bacterial component — that of *Clostridium perfringens* — which has been incriminated in many cases of this syndrome in previously normal people, notably the New Guinea highlanders after pork feasting, sometimes in epidemic proportions, and in sporadic form in cases after meat diets. We studied the occurrence of *Clostridium perfringens* in the affected bowel contents and bowel wall by smear or histology and culture, the serotypes of the isolates and their biochemical activities notably the ability to liberate the vasoactive histamine from histidine, and levels of anti-toxin to the alpha toxin of *Cl. perfringens* in the patients' blood. There was no doubt on the pathogenetic role of this clostridium in our cases. Yet if we were to provide a unified aetiological theory on NE, we had to reconcile it with the multitude of other factors listed in the literature and our postulated immunological origins. It was quite difficult to disentangle and make some sense of the mass of data in the literature on the pathogenesis of this syndrome. The natural temptation was to propose multiple theories to account for them.

Here was a syndrome in need of an aetiological theory. We then had recourse to a basic approach in science. When confronted with a multiplicity of apparently unrelated facts which might breed multiple theories to explain them, it becomes aesthetically satisfying and scientifically sound to wield Occam's razor and reduce that multiplicity to, ideally, a single theory which encompasses all the observed facts.

We proposed that this syndrome with all the documented, diverse aetiological and associated factors, is a single entity, the pathogenesis of which is classifiable into two stages. In Stage 1, a necrotic focus or foci are established in the inner layers of the bowel wall. This is followed by a final common path, irrespective of the aetiology of Stage 1, in Stage 2 in which the ingested or commensal gut clostridia colonise these foci, and exert their well known histotoxic or histolytic reactions which end up as gas gangrene of the intestinal wall. As for the acceptance of this view, it was gratifying that this paper published in 1980, was listed as recommended reading in the Oxford Textbook of Medicine. Let us apply this theory to the diversity of factors which have been associated with this syndrome in its widest spectrum.

One major cause of necrotic foci in Stage 1 is a functional vascular event which leads to intestinal ischaemia, often brought about by the operation of the microvascular shunting mechanisms in the inner layers of the intestinal wall. The jejunal mucosa predominantly, undergoes necrosis and when the circulation is restored, reperfusion-haemorrhage occurs. A recent update on ischaemic intestinal injury and re-perfusion injury is given in the February 1992 edition of The Surgical Clinics of North America. In human neonates, especially the ones stressed by prematurity, respiratory distress, and exchange transfusions, similar reflex shunting, compared with the diving reflex of seals, occurs. In hypotensive adults with traumatic shock, haemorrhagic shock, myocardial infarction, aneurysmal rupture, the same process of vascular shunting operates. In infective states of the intestine, necrotic foci result from the action of exotoxins, endotoxins or Schwartzman reactions. In NE in the natives of New Guinea, the consumption of semi-cooked pork grossly contaminated with *Cl. perfringens* type C with its very potent and necrotising beta toxin, the initial necrosis of the bowel wall is initiated (Stage 1) and continued into frank gas gangrene (Stage 2) by this single aetiological agent, *Cl. perfringens* type C. We then added the group of hypersensitivity reactions to diverse organisms as also initiating Stage 1. The numerous factors which have been described as associated with NE, are in our opinion either capable of initiating the ischaemia and/or necrosis or are capable of merely promoting those events. Other factors are promotive in Stage 2. The terminal event which we could call Stage 3 is irreversible endotoxin shock caused by the entry of bacterial endotoxin through the devitalised intestinal wall.

It was many years later when I attempted to validate our finding that many of our cases of NE were initiated by hypersensitivity reactions of the immune complex type. In a few cases human immunoglobulin G deposits were located with fluorescent anti-human globulin, in the walls of the small arteries which showed leucocytoclastic va-

sculitis. Antigen identification was not attempted nor did we have the fluorescent tracers for the components of complement. Further study was then abandoned because the incidence of cases sharply dropped in the late 1970s and still remains very low. We would still retain our *Ascaris* hypothesis and suggest that this reduction might have resulted from the replacement of the worm paralytic piperazine, with the ascaricidal thiabendazole and pyrantel. Here then is an example of the synergistic role of pathophysiological, bacteriological, immunological, and parasitological factors in the genesis of a single clinical entity, decipherable through an interdisciplinary approach.

Let me now turn away from the laboratory and consider what morals we can glean from the story of Aldo Castellani. I do not think autobiographies are written with a moral in mind. Yet perceptive reader cannot fail to identify those values in science and medicine which he implied in his writings. Let me expand on one of them in answering your hypothetical question which I promised to answer.

This concerns the modern phenomenon of specialisation. In his time the disciplines, as we call them today — parasitology, bacteriology, virology, immunology, pathology and so on — were not in existence. The challenge of disease therefore was a holistic one which demanded the holistic response that Castellani made. The vast explosion of scientific knowledge since his time perhaps one justification now for the identification of these specialities. Another hidden justification is the convenience for administrators. But there is inherently no scientific validation for such a compartmentalisation. Yet that will remain but how should we respond to it? Clearly it demands the promotion of interdisciplinary research, or, more advantageously, of what I call 'internal integration' where the researcher is familiar with the diverse but related fields with which his work is concerned. Allied to this syncretic approach to medicine is the question which I have heard, sometimes asked, 'are clinicians scientists?' If the answer, as I believe, is sometimes a negative one despite the fact that the advance of medicine is a causal or resultant but integral part of the advance of science, the original sin then lies in our faulty approach to education in the medical sciences. There is hardly any exposure of students to the history, philosophy, concepts and methods of science nor to logical, critical and creative thinking.

A second implication of Castellani's writings relates to national development. He recalls the pivotal role of medical research on malaria and yellow fever and its application, for example in having made possible the construction of the Panama Canal as much as having made, in his opinion, a third of the world habitable. The developmental role of science can, I must add, also be

extrapolated to the national scene. Take the Lankan example.

It is anomalous that centres of excellence in tropical medicine are found in Liverpool and London rather than in the tropics themselves. Ironically the anomaly, in converse, still persists in those former colonies, in Sri Lanka for one, where the persistent or increasing threat from malaria, filariasis — and many others, has not yet bred the establishment of similar centres of research excellence in our country except of course, the centre of Professor Kamini Mendis. On the basis of much interdisciplinary research on ARBOviral encephalitis, tuberculosis and leprosy, filariasis, diarrhoeal disease, mycology and agrochemical poisoning, the Peradeniya Faculty proposed the establishment of a Centre for Research in Tropical Medicine (CRTM). After ten years of haggling, a mere 50,000 rupees was granted to set it up. And no wonder, the CRTM still remains a paper dream. Yet it is of some morbid amusement to me that our bodies composed of scientists still hold seminars workshops and learned talk on science for development.

The third moral is concerned with administration of science and scientific institutes. Castellani recounted in his autobiography, that in Naples, his appointment as Professor of Tropical Medicine "...was made in virtue of an old law, according to which the university could call to fill a newly-created chair any man of 'international fame', with *concours* or of any other of the old bureaucratic procedures". Castellani was next recruited, even as a non-British national, as the Director of the Bacteriological Institute in Colombo, the forerunner of today's MRI. Our obsession on the other hand is with 'point schemes' which can lead to recruitment and promotion on minimum qualifications or, to borrow Homi Bhabha's metaphor, at the floor rather than at the ceiling of talent or promise. Castellani makes no explicit statement about administration but one can surmise that he provided benign and effective leadership. I see little evidence of that sort of administration in the scientific institutes in this country in post-independence times.

I am now constrained to contrast that sort of benign and effective leadership, with my increasing conviction which I first expressed publicly in 1980 that the greatest threat to the survival, let alone the growth, of science in this country comes from the scientists themselves. I am often criticised for saying that, so I hope you will forgive me when I quote the following as one of many examples. European scientists collaborating with us are perhaps now on a major advance with a therapeutic immunosuppressant with a novel mode of action, for organ transplantation, autoimmune or hypersensitivity diseases; they are working on a material which we have been researching on for the last twenty years. We ourselves planned a project, designed to investigate its value in these

situations. If that therapeutic immunosuppressant becomes a reality, Lanka would have lost the claim to priority because this project was sabotaged through a classic example of deliberate maladministration in science, — a saddening insult to the memory of Aldo Castellani, who, as Professor Kasuke Ito wrote, "...also had the ability to involve others in his enthusiasm, which I think was one of his greatest attributes".

I will conclude by saying that our common goal, your college's Madame President, and mine is to make some contribution to the advance of medical science in this country. I must congratulate the Ceylon College of Physicians for having instituted the Castellani memorial lecture, so emphasising the benefits of an interaction between the clinical and basic sciences in pursuing that goal. Your College has thereby and in effect responded to the question which Castellani asked in concluding his autobiography — "Can there be a worthier ambition than to add a new stone, however small, to the never-completed edifice of knowledge"? I hope I have in this lecture, illustrated the symbiotic role of the basic sciences with clinical practice and research in adding those new stones to the edifice of medical science.

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