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THE FIFTH DR. P. V. GHARPURE ORATION

Hepatitis Virus Infection—Global Concern

N. C. NAYAK

Professor of Pathology

**All India Institute of Medical Sciences,
New Delhi.**

Standing before you here I feel justifiably honoured to have been given this opportunity of paying my personal tribute to this great Institute of work and learning and to Dr. P. V. Gharpure, one of the chief contributors to its glorious heritage. I am extremely grateful to the Haffkine Institute, its Governing Body and Director for the honour they have bestowed on me.

Dr. Purshottam Vishwanath Gharpure born in the Victorian era, was one of the pioneers in the field of Virology in this country. Dr. Gharpure, a brilliant student of pathology and microbiology, was the recipient of numerous coveted awards and fellowships during his career as a student at the Grant Medical College, Bombay. Later on he held the post of the Professor of Pathology & Microbiology at the same college from 1925 to 1951. During the second world war he served in the Indian Army as a Major.

Dr. Gharpure was instrumental in establishing the Enterovirus Research Unit of the ICMR. Originally the unit, then known as Polio Research Unit was operating from the Grant Medical College where Dr. Gharpure was employed as Professor. Later on the unit was transferred to Haffkine Institute with an

enlarged scope and expanded activities to cover the entire group of enteroviruses. His research work in the field of enteroviruses has received international recognition. It was his initial efforts with the WHO that ultimately led to the establishment of a laboratory for production of Polio vaccine, now with the Haffkine Bio-Pharmaceutical Corporation.

My first contact with Dr. Gharpure was more than two decades back when I was entering the portals of pathology as a special discipline. As a young member of the profession as well as later, one could not but admire Dr. Gharpure's keen and brilliant mind, logical, scientific attitude and a mild, generous disposition. Dr. Gharpure's contributions to medicine in general and to pathology and microbiology in particular have been many and he will always be remembered as one of the doyens in medical sciences in our country.

In paying my personal homage to this great scientist I wish to briefly highlight this morning, some areas of investigative work by my colleagues and myself on hepatitis virus infections, a field that has made an impact on human health and disease possibly unprecedented in history. Man's success in mastering the

*Delivered at Haffkine Institute, Parel Bombay-400 012 on 24th Feb, 1981.

microbes lies in his ability to combine astute observation, technological skill and logical deduction. As Louis Pasteur said "To be astonished at anything is the first movement of the mind towards discovery". Within one and a half decade of Blumberg's discovery of the Australia Antigen we know that at least three major categories of hepatitis viruses infect man. Knowledge on the structure and biological properties of these viruses, the diseases associated with them, and the means of identifying and possibly preventing infections are accumulating at an unbelievable speed. By using various immune markers it has been possible to relate certain forms of liver diseases with these virus infections.

The B virus having been the first to be discovered, is the one, most extensively studied. Various components and antibodies to each one of these have been identified. Serological studies have shown that the hepatitis B virus (HBV) is a highly successful agent in terms of its symbiosis with the host. Not only but also a very peculiar, often prolonged but also a very peculiar, often prolonged asymptomatic carrier state, frequencies of occurrence of which can be extremely high. A recent estimate reveals that in the USA alone about 15,000 individuals suffer from acute hepatitis B annually. The figure for our own country is certainly several fold more, possibly numbering to millions, though no accurate or even rough estimate is available. The number of asymptomatic carriers, on the other hand, is computed to a staggering figure of 200 millions in the world today, comprising of approximately one-twentieth of the total population. The carrier rates, however, vary from one part of the world to another, being very high to the tune of

15 to 20% in the Far East and Africa, 4 to 10% in India, Southern and Eastern Europe and the USSR and low figures ranging from 0.1 to 0.7% in the UK, Western and Northern Europe, USA, Canada, South America and Australia.

Our own interest in the relationship of HBV infection to chronic and fatal liver diseases was initiated almost at the same time when Prof. Shikata was reporting on orcein positivity of the surface component of the virus (HB s Ag). The studies I am going to describe now were carried out by a young intern, Dr. Rajiv Sachdeva during her period of elective posting and by Dr. Asha Dhar. We initially posed the following two questions to ourselves: (1) if the formalin fixed antigen can be stained by a non-specific histochemical reaction like orcein staining, can it also be identified by more specific immuno-histochemical tests? (2) If the answer to (1) is "yes", can one define the degree of specificity and reliability of the technically simpler orcein stain? Using conventional formalin-fixed paraffin-embedded liver tissue, some of these stocked for several years, it was possible to clearly localize and identify the HBsAg by indirect immunofluorescence and indirect-immuno peroxidase staining reaction. Also, complete locational and morphologic identity was seen between material stained by these specific immunological methods and by orcein. Since immuno-staining revealed only the surface coat of the virus this seemed to be the only component stainable by orcein. Applying identical immunohistochemical reactions after using specific antibody to the core component of virus B (HBsAg) we could again successfully demonstrate this antigen in the nuclei of hepatocytes in conventional

formalin-fixed paraffin embedded tissue. Both components of the virus showed complete randomness in distribution within the liver and inside the liver cells. This latter finding made it apparent that detection or otherwise of HBV components in a sample of liver tissue would depend entirely on the quantum of material examined. We later confirmed this possibility by comparing positivity rates between needle biopsy, wedge biopsy and autopsy material as well as between increasingly larger amounts of tissue examined for the virus. These findings helped us to draw the following conclusions. Application of these immunological techniques and orcein staining to material stored for decades in hospitals and laboratories and available for examination of relatively large samples from each specimen would provide fairly conclusive evidence on the association or otherwise of hepatitis B virus in a variety of liver diseases.

We next proceeded to test the hypothesis that hepatocellular cancer (HCC — primary liver cell cancer) and certain forms of hepatic cirrhosis in man are associated with hepatitis B virus infection. One of the important and first requirements to ascribe causal role for a virus to a tumour is association between the two both at epidemiological and at biological levels. Sufficient indications for the former are already available. Parts of the world where carrier rates for HBV are high show an extremely high incidence of HCC as well as of non-alcoholic cirrhosis of liver. In fact, the frequencies of occurrence of these fatal liver diseases in different parts of the world can be directly correlated with the respective HBsAg carrier rates. Also, HCC almost universally occurs in a liver that is cirrhotic as

well, particularly of the non-alcoholic variety. With this background of epidemiological informations it would be necessary to establish a biological association between the virus and these forms of liver disorders. Serological marker studies have shown evidence of continuing infection by HBV in significant numbers of these categories of patients as compared to controls. We, therefore, wanted the following questions to be answered while trying to identify viral components in liver tissue: (1) Is there a significant association between the virus on one hand and HCC and non-alcoholic cirrhosis on the other? (2) If so, how strong are these associations? Initially we collected from different parts of the country 50 cases of hepatocellular carcinoma, 58 of cirrhosis and 54 of miscellaneous liver disorders, the last named serving as controls. The staining procedures were rigidly controlled and particular attention was given to screen adequate amounts of tissue in each case in order to overcome false negative results due to sampling errors referred to earlier. HBsAg was detected in 94% of HCC, 71% of cirrhosis and only 2% of control livers while HBsAg was found in 22%, 31% and none respectively. Both antigens were present in 9 of 41 HCC, 12 of 39 cirrhosis and none of 25 controls. HCC was associated with cirrhosis in the majority (92%) and antigen positive cirrhosis had a higher chance of harbouring HCC than antigen negative disease. In 4 cases HBsAg was seen inside occasional tumour cells though no tumour examined a much larger number of livers our showed HBsAg. We subsequently removed at autopsy, 904 to be precise, through the aegis of the International Agency for Research on Cancer as coordinator in an international collabora-

tive programme. While this material showed patterns of positivity in HCC and in non-alcoholic cirrhosis more or less identical to those in our initial investigation, an additional feature that emphasized the association between HBV infection and these two liver disorders was the fact that a further correlation was observed between the positivity rates and the risk of developing HCC. In other words, livers obtained from high and intermediate cancer areas showed much higher frequencies of positivity for HBsAg as compared to those from low risk areas. Also, the commonest histological variant of HCC, namely the trabecular type was most frequently associated with HBsAg in the liver. In this material again, livers with cirrhosis not only tended to be more frequently positive for HBsAg but also in the latter's presence harboured the tumour more often than in its absence. We concluded that not only is there a significant association between hepatitis B virus, HCC and cirrhosis but also that these associations seem to be very strong indeed. We surmised that infection with HBV may lead on to the development of hepatocellular carcinoma through more than one pathway and that cirrhosis may provide a rich soil for the development of this fatal tumour.

Another requirement for proving virus as an etiological agent in cancer is to demonstrate a direct target cell transformation by the virus itself. There are essentially two ways of looking at this aspect of the problem, firstly to look for neoplastic features in cell transformed by the virus and secondly to demonstrate virus production by human tumour cells which grow *in vitro* like cancer cells while maintaining features of the cell of origin.

We were lucky in being able to establish and maintain a human hepatocellular carcinoma cell line which releases hepatitis B virus surface antigen to the medium. This work was largely carried out by my colleague Dr. P. K. Das. The cell line, referred by us as DELSH-5 was derived from a HBsAg sero- and tissue-positive patient subjected to operative wedge biopsy of the liver in which the cells not only resemble hepatocytes on light and electron microscopic examination but also possess biosynthetic markers of the latter like albumin and alpha foeto protein. They have an aneuploid karyology with the major modal chromosome number being 61, and have been releasing moderate amounts of HBsAg following the few initial passages. Immuno-histochemical techniques as well as electron microscopy on the other hand, have consistently failed to demonstrate any virus component inside the cell. We have never been able to show HBsAg either inside the cell or in the medium. With the availability of cell lines like this it would be possible to study several aspects of hepatitis B virus liver cell interaction with particular reference to the oncogenic potential of the virus. In fact, some recent studies have indicated that parts of the viral genome are incorporated in the chromosomes of these tumour cells and seem to be responsible for the coding of the RNA protein representing HBsAg. Certain other criteria still need to be fulfilled in order to finally say with definitiveness that HBV does induce hepatocellular cancer in man.

One other aspect of hepatitis virus infection that has interested us is the pathogenesis of the liver cell injury in acute and chronic hepatitis. There is almost no doubt that the virus itself, and

this is particularly obvious in case of the B virus, has no cytopathic effect. Carriers often contain a very large load of HBV components inside the liver cells without exhibiting any significant liver injury. Even when one detects the viral components in livers of chronic hepatitis, cirrhosis or HCC the individual cells that contain the virus frequently show no evidence of injury or inflammatory cell reaction whatsoever. Compelling evidence is available now to indicate that out of the two limbs of immune reaction mounted by the host against the virus the cellular rather than the humoral one plays a major role in the pathogenesis of hepatocytic injury. Indeed it is this immune killing of the infected liver cell that is the body's principal attempt at eliminating the virus. Lymphocyte is the predominant reactant cell in the livers of acute hepatitis, often seen in close contact with infected cells while *in vitro* tests have demonstrated heightened cellular immune reactions mediated by lymphocytes against purified hepatitis B virus exhibited as blast transformation or killing of antigen coated cells. However, very recent studies reveal that the specificity of liver cell killing is not determined by recognition of hepatitis B viral components by sensitized lymphocytes. It is thus possible that these effector cells identify either some constituents of the normal cell membrane or some new or altered antigens expressed in the infected liver cell.

In this connection a great deal of interest has recently been shown in the so-called "liver specific protein" (LSP), a substance or substances which appear to be specific to the liver cell membrane and can be referred to as liver membrane protein (LMP). In our laboratory Dr.

Jain, Dr. Panda and Miss. Pranava have attempted to examine the role of immunological reaction against LSP (or LMP) in liver cell injury, both in human patients of hepatitis as well as in experimental models. Antibodies to heterologous LSP was looked for by indirect immunofluorescence test on normal rabbit or rat hepatocytes and by hemagglutination of sheep red blood cells coated by purified rat LSP in sera of patients suffering from acute hepatitis taken at different phases of illness. Cell-mediated cytotoxicity was assessed by studying killer action of lymphocytes from these patients and from controls on isolated and short-term cultured rat liver cells. Our results showed high levels of cytotoxicity in 39 of 57 patients, the values ranging from 1% to 68% as compared to 1% to 13% in controls. Mean cytotoxicity during the first three weeks after jaundice was $35.5\% \pm 12.8$ in contrast to $5.6\% \pm 4.5$ in the controls. Cytotoxicity values even at the onset of the jaundice were higher in all patients when compared to the upper limit of controls. These high toxicity started reducing after 3 weeks and touched near normal at 6 weeks and later. Serial studies done in 14 patients showed an identical pattern and out of 3 patients who continued to show high levels of cytotoxicity after 6 weeks, one later developed chronic active hepatitis. HBsAg seropositivity did not relate with cytotoxicity. Also, these values did not relate to the serum transaminase and bilirubin levels. The two tests for humoral immune response against LSP correlated well to the extent of 80.5%. Significant positivity was seen only by the 4th week after onset of jaundice and by 6 weeks and later a decline in the titre of antibodies and in the number of patients showing detectable antibody levels was noted.

Serial studies on antibodies again showed identical pattern. Since it is well known that by the time the patient of acute hepatitis presents in the hospital, hepatocellular degeneration and necrosis is not only well established but often is on the decline, the exact implication of the immune reaction findings are difficult to assess. It is possible that higher levels of cytotoxicity may have been manifest in the earlier prodromal phase of the disease. It is also possible that the immune reactions observed in our patients to LSP may represent an epiphenomenon unrelated to initial hepatocellular damage.

We have recently studied the immunological response of mice to LSP with essentially three questions in mind. Firstly, what is the type of immunological response mounted against LSP? Secondly, does liver injury and inflammation of the type seen in acute or chronic hepatitis occur in the sensitized animals? And lastly, is one or more immunological response related to the liver injury? Purified rat LSP was injected in one or two doses to batches of mice and development of cellular and humoral immunity were tested at variable intervals initially extending upto 8 weeks but in subsequent experiments to 16 weeks. Delayed type hypersensitivity (DTH) measured by foot pad swelling following a small challenge dose and cellular cytotoxicity by lymphocytes on hepatocytes both in autologous and homologous *in vitro* systems served as parameters of cell mediated immune reaction. Humoral immunity was tested by looking for antibodies through precipitation reactions and hemagglutination of sheep red blood cell coated by purified rat LSP. Significant DTH compared to the controls deve-

loped in the sensitized animals at 2, 3 and 4 weeks 24 and 48 hours after challenge. Not only was there obvious swelling of the test foot pad as compared to the control injected by buffer alone, but also the challenge tissue was densely infiltrated by lymphocytes, histiocytes and neutrophils. In the autologous system significant cytotoxicity was elicited in the sensitized animal only, while in the homologous system no cytotoxicity was observed when sensitized hepatocytes were put with control animal's lymphocytes though some degree of cytotoxicity was apparent when control hepatocytes were incubated with lymphocytes from sensitized animals. These experiments were designed to eliminate possible interference of HLA incompatibilities in these test systems. The cytotoxicity appeared some what later than the DTH at 4th week, gradually increased through 6th and 8th week and attained a maximum by the 14th week, plateauing at 16th week. DTH on the other hand reduced by 6th week.

Examination of the liver showed focal infiltration by lymphocytes and a few histiocytes in the initial phases without any significant liver cell damage but at 14 and 16 week almost all animals showed distinct ballooning degeneration, necrosis and severe diffuse inflammation of the liver by lymphocytes, phenomena in many ways reminiscent of the acute hepatitic damage in human liver. While the findings in this study are extremely interesting and challenging, the observations are still preliminary and need further substantiation. It, however, appears that immunological response against LSP may be crucial in the pathogenesis of liver injury of acute and chronic hepatitis.

It is apparent that while great strides have been made in the understanding of infections by hepatitis viruses, a lot remains to be explored. In the words of the late John F. Kennedy : Life is unfair. No sooner do you get one problem solved than another one, often worse, rises up to take its place. The rates at which humans get infected by the different types of hepatitis viruses is appalling. Hepatitis A virus infects almost all individuals throughout the world by the age of 20 years. The B virus is phenomenal in its existence and infective potential, entering through many routes into its human host. The non-A non-B viruses now constitute the most important agent for post-transfusion hepatitis in the West. A very large number of acute hepatitis occurring every large share of chronic hepatitis, cirrhosis and hepatocellular cancer are most likely day the world over and undoubtedly a related to these virus infections. When world figures for HCC are computed,

being a frequent cancer in the more populous part of the world, this cancer, a very fatal one, possibly comes out as the number one cancer in the human male. Obviously the concern regarding infection by hepatitis viruses is grave and is universal. Luckily, new light is being thrown on many aspects of these infections every day, more is likely to be learnt about each one of these viruses, particularly the newer ones like non-A non-B virus. The vaccine against hepatitis B virus is almost ready for mass application. Very current reports indicate that it is not only effective but also entirely safe providing in addition a wide time range for protection. There is then light at the end of the tunnel and preventive measures, understanding of the basic pathogenesis of the various lesions produced by the viruses, and application of newer therapeutic regimens would certainly be able to save to a great measure the human race from this particular menace.

Prevalence of Multi-drug Resistant Coliforms in Human Gut : Effect of Antibiotic Therapy

V. M. RANGNEKAR, S. MUKERJI, S. V. GADRE and D.S. CHITNIS

Haffkine Institute, Parel, Bombay-400 012.

Increasing selective pressure mainly imposed by indiscriminate use of antibiotics is one of the main factors responsible for emergence and persistence of drug resistant faecal coliforms. The present report depicts the effect of antibiotic therapy on the incidence of multi-drug resistant faecal coliforms in individuals from local population in Bombay. Patients being treated with antibiotics showed higher incidence ($p < 0.05$) of multi-drug resistant and R+ coliforms in comparison with patients not undergoing antibiotic therapy at the same hospital. This incidence was considerably greater than that in healthy individuals not treated with antibiotics for one month. Healthy individuals from Bassein village with no exposure to antibiotics as therapeutics for six months showed least incidence (6%) of drug resistant coliforms. Resistance to the drug spectrum — Sulphadimidine, Ampicillin, Streptomycin, Tetracycline and Chloramphenicol was most prevalent and plasmid borne. Large proportion (81.0%) of coliforms from the gut of typhoid patients carried R plasmids coding chloramphenicol resistance. Co-trimoxazole resistance was detected in 8 *E. coli* cultures. The role of antibiotic treatment in selecting a predominantly multi-drug resistant coliforms flora in the human gut is discussed in the light of above observations.

Introduction

R-plasmid mediated Multiple Drug Resistance (MDR) has become a notorious feature of faecal coliforms in recent years¹. Such coliforms complicate treatment when they cause endogenous or nosocomial infection^{2,3,4}. Moreover, they can transfer their drug resistance to potential enteric pathogens such as *Salmonella typhi* and this implicates a serious problem³. Increasing selective pressure mainly imposed by indiscriminate use of antimicrobials is one of the main causes for emergence and persistence of drug resistant faecal coliforms^{6,7}. The present study depicts the effect of selective

Pressure exerted by antimicrobials on the incidence of drug resistant coliforms in individuals from local population in Bombay.

Materials and Methods

Faecal samples from healthy and hospitalised individuals were plated on MacConkeys agar and predominant colony types were identified by standard biochemical tests. The isolates were then subjected to antibiotic sensitivity test by the disc diffusion technique of Bauer *et al* and multidrug resistant isolates were further screened for conjugal transferability of MDR by the

method of Watanabe⁹. After mixed cultivation of multidrug resistant isolate (prospective donor) with *Escherichia coli* K12 Nal^r (prospective plasmid-negative recipient), aliquots of mating mixture were plated on MacConkeys agar containing nalidixic acid (Nal) and donor drug markers to select transconjugants. Plasmid nature of drug resistance was confirmed by its transferability to *E. coli* K12 Nal^r in primary crosses and further to *E. coli* K12 c 600 Rif^r in secondary crosses.

Results

On the basis of history of antibacterial therapy individuals were classified into six different groups. The incidence of multidrug resistant and R+ enterobacteria in each group is presented in Table I.

Group I consisted of 205 hospitalized patients with respiratory and urinary tract infections, subjected to antibacterial therapy. Of these, 105 were adults and 100 were children. The bacterial spectrum of predominant isolates is not presented in table, however, 181 were *E. coli* 36 were *Enterobacter* species and 3 belonged to *Proteus* species. The incidence of multidrug resistant and R+ isolates was 50.8% and 25.8% respectively in adults, and 80% and 33% respectively in children. Group II comprised of 30 patients at another hospital undergoing antibiotic therapy for respiratory tract infections. The incidence of R+ coliforms was higher ($P < 0.05$ by chi-square method) in this group in comparison with that in Group I.

Group III included 37 culture positive cases of typhoid. These were admitted to an infectious diseases hospital and were under chloramphenicol therapy. Multidrug resistant coliforms were iso-

lated from faecal samples of 34 cases and 30 of them possessed R plasmids coding chloramphenicol resistance together with other drug resistance markers. However, none of the *S. typhi* isolates from blood samples of these patients were resistant to chloramphenicol. Group IV consisted of 41 patients hospitalised for hypertension or other physiological disturbances and were not given antibiotics. This group was included in the study to see the effect of hospital environment on bacterial colonization of the human gut. The incidence of multiple drug resistance in this group of individuals was relatively lower ($P < 0.05$) than that in Groups I-III.

Group V composed of 543 normal adults and 100 normal children who had not consumed any antibacterials atleast in the past one month. The faecal isolates consisted of 653 *E. coli* 13 *Enterobacter* species and 4 *Proteus* species. The incidence of R+ isolates was 15.14% in adults and 6% in children and this is significantly lower ($P < 0.05$) than any of the above groups. Group VI comprised of 100 individuals from Bassein village who had not visited their family doctor for atleast 6 months. This population was expected to have least exposure to antibiotics and was therefore selected for comparative study. Only 2% of the faecal coliforms from this group were R+. Several patterns of MDR and transferable MDR were demonstrated in isolates from each group (Table II). The incidence of MDR was relatively higher than that of resistance to a single drug. Resistance to the drug spectrum — Sulphadimidine (Su), Streptomycin (Sm), Ampicillin (Ap), Tetracycline (Tc) and Chloramphenicol (Cm) was most prevalent. All isolates resistant to Kana-

Table I

Incidence of multidrug resistant and R+ enterobacteria in different population groups

Group	Population	Antibiotic treatment	No. of individuals	No. of isolates	No. of isolates with MDR	No. of R+ isolates
I	Hospitalized patients with respiratory and urinary tract infection	one or more drugs				
	Adults		105	120	61 (50.8)	31 (25.8)
	Children		100	100	80 (80.0)	33 (33.0)
II	Hospitalized patients with respiratory tract infections	one or more drugs	30	30	25 (83.3)	19 (63.3)
III	Hospitalized typhoid patients	chloramphenicol	37	37	34 (91.9)	30 (81.0)
IV	Hospitalized patients with physiological disturbances	None at hospital	41	45	17 (37.7)	10 (22.2)
V	Healthy individuals	None for one month				
	Adults		543	570	174 (30.5)	84 (15.4)
	Children		100	100	12 (12.0)	6 (6.0)
VI	Healthy individuals from Bassein village	None for six months	100	100	6 (6.0)	2 (2.0)

Figures in parentheses show percent values.

Table II

Pattern of MDR and en bloc transferable MDR in isolates from different groups of individuals

Pattern of MDR	No. of Gr. I	isolates Gr. II	from Gr. III	different Gr. IV	population Gr. V	groups* Gr. VI
Su-Sm-Cm-Ap-Tc-Km-Nm Su/Tp	3(0)	0	0	0	0	0
Su-Sm-Cm-Tp-Tc-Su-/Tp	1(0)	0	0	0	0	0
Su-Sm-Ap-Tc-Su/Tp	4(0)	0	0	0	0	0
Su-Sm-Cm-Ap-Tc-Km-Nm	28(17)	4(3)	0	1(0)	29(15)	1(0)
Su-Sm-Cm-Ap-Tc-	69(27)	11(11)	22(20)	14(9)	66(37)	3(2)
Su-Sm-Ap-Tc	16(0)	2(0)	2(2)	1(1)	16(4)	0
Su-Cm-Ap-Tc	4(4)	0	0	0	6(6)	2(0)
Su-Sm-Cm-Tc	0	4(3)	9(9)	1(0)	0	0
Su-Ap-Tc	3(0)	0	0	0	1(1)	0
Su-Sm-Ap	7(0)	0	0	0	9(0)	0
Su-Sm-Tc	4(2)	4(2)	0	0	1(0)	0
Sm-Ap-Tc	0	0	0	0	1(0)	0
Ap-Cm-Tc	0	0	1(0)	0	0	0
Su-Sm	1(0)	0	0	0	16(0)	0
Su-Ap	1(0)	0	0	0	9(0)	0
Su-Tc	0	0	0	0	3(3)	0

Figures in parentheses show no. of isolates with MDR, transferable en bloc during conjugation.

*Population groups numbered as in Table I.

mycin (Km) were also resistant to Neomycin (Nm) and this linkage was maintained even during conjugal transfer of drug resistance to *E. coli* K12. Eight cultures of *E. coli* from hospitalized children harboured non-transferable resistance to Co-trimoxazole (Su/Tp) in addition to other drugs.

Discussion

The present study reveals higher incidence of MDR coliforms in the gut of patients being treated with antibiotics in comparison with that in patients not undergoing antibiotic therapy at the same hospital. Administration of a single drug such as Chloramphenicol or Tetracycline, led to emergence of coliforms carrying resistance not only to these antibiotics but also to other commonly used drugs. This clearly reflects the role of antibiotics in effectively selecting a predominantly multidrug resistant flora in the gut. It is alarming to note that resistance to the drug spectrum-Sulphadimidine, Streptomycin, Ampicillin, Tetracycline and Chloramphenicol, which includes most of the commonly used antimicrobials was prevalent and plasmid-borne. However, the frequency of isolation of R+ coliforms is considerably lower than that observed by Panse and Wadwa¹⁰ in Pune, Panicker¹¹ in Calicut, Smith and Halls¹² in Britain, Whithead¹³ in Bristol and Moorhouse¹⁴ in Ireland. This could possibly be due to differences in populations screened, for in the present study itself Group I and Group II, which comprised of patients at two different hospitals, differ markedly in incidence of R+ coliforms.

Besides, it is likely that in some of our isolates the R plasmids might not have been detected in conjugation experiments, either because of their poor transferability

to *E. coli* K12 recipient or due to lack of functional transfer systems for the resistance genes.

Hartley and Richmond⁶ observed that after termination of antibiotic therapy, that is, after selection pressure had been withdrawn, the coliform flora of the gut became predominantly drug sensitive; the time course, however, varied from 3 days to 8 months or more. They concluded from their study that in the period following termination of antibiotic treatment the ability of resistant coliforms to persist depends upon their capacity to colonize the gut. The drug resistant bacteria do not seem to have any advantage over their drug sensitive counterparts in a drug-free environment. The persistence of multi-drug resistant coliforms in the gut of individuals from Group V and Group VI (who had undergone antibiotic therapy one and six months or more, respectively, before stool culture) could, perhaps, be an indication of their colonizing ability. Nevertheless, low levels of antibiotics in the environment might have also contributed to their persistence.

It is highly disquieting to note that large proportion of multidrug resistant coliforms from the gut of typhoid patients carried transferable chloramphenicol resistance. In view of the endemicity of typhoid in our country^{15,16} this poses a danger of transfer of chloramphenicol resistance to *S. typhi* thereby rendering chloramphenicol ineffective in the treatment of typhoid. The occurrence of co-trimoxazole resistance in *E. coli* is also alarming, for this relatively new synergistic combination¹⁷ has proved extremely effective in combating infections where other antibiotics had failed. Moreover, this drug has been successfully used in

typhoid cases and it worked excellently in typhoid outbreaks caused by chloramphenicol resistant *S. typhi* strains¹⁸. Development of resistance to newer antibiotics seems to be an unending phenomenon and if this problem remains unresolved, we may be thrown into the 'pre-antibiotic era', for authorities are pessimistic about the discovery of an entirely new type of drug to which resistance cannot develop¹⁹. Besides, research at molecular level aimed at combating drug resistance has not yet materialized. Hence prevention of inde-

criminate use of antimicrobials seems to be the only reasonable approach towards preserving the usefulness of the present day drugs.

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A Trial of Counterimmunoelectrophoresis Test for the Detection of Antibodies in experimental and Human Hookworm Infection

M. K. BHOPALE, SUSHILA MENON and VIJAYALAXMI R. KAMATH

Department of Zoonosis, Haffkine Institute, Parel, Bombay - 400 012.

Sera samples from the infested hookworm patients, experimentally *Necator americanus* infected rabbits and *Ancylostoma caninum* infected mice were analysed for the detection of precipitin antibodies by counterimmunoelectrophoresis. The antigens were prepared from larval and adult *N. americanus* (Rabbit strain) and larval *A. caninum* (Dog strain). This test revealed the presence of precipitin antibody in 9 samples out of 45 in infected rabbits 2 samples out of 7 samples of human hookworm patients using *N. americanus* larvae and adult worm antigens; 6 samples out of 18 in infected mice using *A. caninum* larval antigen during the observation period. Cross reaction was also observed between *N. americanus* and *A. caninum* antigens. Experimental results suggest that this test has limited use in the immunodiagnosis of hookworm infection.

Introduction

In recent years, counterimmunoelectrophoresis (CIEP) test has been adapted for the serodiagnosis of a wide variety of helminthic infections like trichinosis (1), filariasis (2), toxocariasis (3), schistosomiasis (4) and fascioliasis (5). In hookworm infection, many immunodiagnostic tests have been used (6-13) but counterimmunoelectrophoresis, to our knowledge, has not been attempted. Present communication deals with the CIEP test results obtained from the hookworm infected rabbit model in which *Necator americanus* behaves as in human beings (14, 15), along with patient's sera using *N. americanus* larval and adult antigens from the rabbit source. Larval ancylostomiasis study was done in another host system e.g.

Ancylostoma caninum in mouse for the confirmation of this test.

Materials and Methods

Infected *N. americanus* (RSG 1-3) rabbits (14), and mice (25 gms body weight) each was orally infected with 1000 *A. caninum* larvae (16) were bled at different intervals for sera collection as indicated in Table. Sera samples from hookworm patients, supplied by J. J. Hospital, Bombay and three samples from helminthic negative healthy persons were used for CIEP. A comparative study for the different serological responses like indirect haemagglutination (17), gel diffusion (18) and CIEP were done for evaluating CIEP test in *N. americanus* infection using rabbit as an experimental model and use of the antigenic materials

from the rabbit source. Different hookworms like *N. americanus* larvae or adult worms (Rabbit source) and *A. caninum* larvae (dog source) were collected. Antigens were prepared (17), protein concentration measured by Lowry's method (19), adjusted to 1.4 mg/ml and stored at -20°C, when not used.

Counterimmunoelectrophoresis was performed on a microscopic slide (7.5 x 2.5 cms). The glass plate was covered with 3 ml. of 1% Agarose (BDH) or 1% Noble agar (Difco) in veronol buffer (pH 8.4, 0.05/u) to which merthiolate (1:10000) was added. The same buffer was used for the electrophoresis. On each plate 6 pairs of wells, 2 mm in diameter and 5 mm apart were cut out in parallel rows. The sera samples were placed in the anodic well and the antigen in the cathodic well. Electrophoresis was carried out at 10°C with constant voltage (100 volts) of 2.2 mA/cm for one hour. These plates were kept at room temperature for 15 minutes after the completion of electrophoresis. Later on these plates were washed with several changes of saline, dried, stained with amido black (1%) followed by washing in 2% acetic acid solution and finally dried at room temperature.

Results and Discussion

The result showed that precipitating antibodies to *N. americanus* larval antigen first appeared in the serum of rabbits 4 weeks after infection and thereafter continued irregularly up to 120 days. Adult worm antigen also showed positive reaction with the sera samples from the infected rabbits. In all, total 5 out of 33 sera samples during the prepatent period (7-60 days) and 4 out of

12 samples during patent and post patent period (60-200 days) were positive with either larval or adult antigens (Table). Most of the sera samples reacted only to larval antigen, others to antigen derived from adult *N. americanus* but none of them to both antigens.

Out of seven hookworm patient sera only two were positive with larval *N. americanus* antigen. However, these samples were negative with adult *N. americanus* antigen by CIEP.

In mice sera samples, positive reaction was observed with larval antigen of *A. caninum* during 3 weeks of infection period but a negative reaction during 4th and 5th week of the infection. These sera samples were also positive with larval and adult *N. americanus* antigens.

Six *N. americanus* infected rabbits showed a remarkable decrease in body weight of 1020 gms (1320 gms), increase in eosinophilic counts of 18.0% (6.0%), decrease in Hb gm% of 12.4% (16.6%) and epg of 45000 (nil) at patent period of 70-80 days post infection as compared with the values of uninfected control animals given in parentheses. These symptoms remained prominent even on the day 140 except the absence of eggs in the pellets. Sera samples from the infected animals were negative to gel diffusion and CIEP test giving low haemagglutinating titre of 1:8 in infected rabbit sera in both patent and post patent intervals with negative results in uninfected control sera samples.

Although the precipitating antibody to *N. americanus* antigens was present in the sera of both rabbits exposed to *N. americanus* and human infected

Table I

CIEP reactions between hookworm antigen and sera collected from infected animals and man

Host (Total number)	Infection	Antigen	Infection period (days)				Unknown period	Positive sera samples (Total sera samples)	Control Uninfected sera samples (Total sera samples)
			7-30	31-60	61-120	120-200			
			Positive sera samples (Total sera samples)						
Rabbit (55)	<i>N. americanus</i>	<i>N. americanus</i> larvae	0(13)	2(20)	2(5)	0(7)		4(45)	0(10)
		<i>N. americanus</i> adult	2(13)	1(20)	0(5)	2(7)		5(45)	0(10)
Human (7)	Hookworm	<i>N. americanus</i> larvae					2(7)	2(7)	0(3)
		<i>N. americanus</i> adult					0(7)	0(7)	0(3)
Mouse (30)	<i>A. caninum</i>		7-35						
		<i>A. caninum</i> larvae	6(18)					6(18)	0(10)
		<i>N. americanus</i> larvae	5(18)					5(18)	0(10)
		<i>N. americanus</i> adult	5(18)					5(18)	0(10)

with it, could be detected only in a limited number of sera samples. The relationship between the degree of infection and antibody elicited is not determined from the present study.

Present study reveals that CIEP test may have very limited application in serodiagnosis of hookworm infection. This may be due to delayed or incomplete antibody production in hookworm patients as suggested by earlier workers using other tests (9,12). Earlier literature suggests that Desowitz (7) was unable to detect antibody in sera of six children

who were heavily infected with *N. americanus* using larval antigen in gel diffusion. Also, Soulsby and Gilles (13) have reported the production of haemagglutinating antibody in children after a lapse of two years. Williams (8) on the other hand, stated that adult *N. americanus* antigen was reactive with sera of two out of six adult patients harbouring *N. americanus* infection. These earlier reports support our views in the present study of *N. americanus* infection in rabbits.

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Synthesis and Study of 2-Substituted-4-Methyl-5-(β -Hydroxyethyl)-6-Hydroxy Pyrimidines as Possible Antimalarial Agents. II.

D. S. SANGHAVI, D. T. CHAUDHARI and P. P. GUDADHE

Department of Chemotherapy, Haffkine Institute, Parel, Bombay - 400 012.

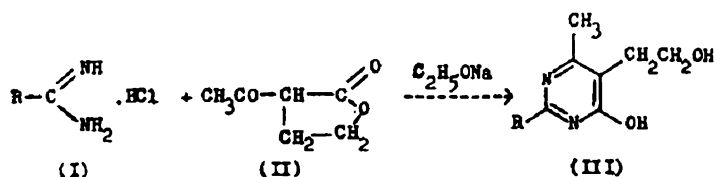
In view of the reported antimalarial activity by 5, 6-substituted pyrimidines, various new 2-substituted 4-methyl-5-(β -hydroxyethyl)-6-hydroxy pyrimidines have been synthesized. Some of them were screened for *in vivo* antimalarial activity against *P. yoelii* in mice.

Introduction

It has been observed that the substitution at 5 and 6-position in pyrimidine ring enhances antimalarial activity. Falco and colleagues (1) synthesised and tested a large number of structural variations of pyrimidine compounds for antimalarial activity. Although, several 2, 4-diamino-5-alkoxy pyrimidines such as 5-ethoxy, butoxy and octyloxy homologs showed activity, the substitution at 6-position also gave, in general, the active compounds in this series (1).

Position 5 in the pyrimidine ring, compared to position 2, 4- or 6 possesses enhanced electron density and permits ready electrophilic substitution. Hence pyrimidine containing different substituents in position 5 have attained great pharmacological importance.

It was, therefore, planned to synthesise pyrimidine compounds in which 2 and 6 positions are substituted by β -hydroxyethyl and hydroxy groups respectively to get 2-substituted-4-methyl-5-(β -hydroxyethyl)-6-hydroxy pyrimidines (III).



where,

R = 4-ClC₆H₄; 4-BrC₆H₄; 4-OC₃H₇C₆H₄;
3,4-(OC₂H₅)₂C₆H₄, etc.

4-Pyridyl, 3-pyridyl, 2'-aryloxy pyridyl.

Chemistry

These pyrimidines (III) were prepared by the interaction of α -acetyl- β -butyrolactone (II) and respective amidines (I) in presence of sodium ethoxide (2). The amidines were obtained from the appropriate substituted nitriles (3). The required α -acetyl- β -butyrolactone was synthesised starting from ethylacetoacetate and ethylene oxide (4) and all substituted nitriles were prepared by the known methods.

Experimental

2-(4'-Chlorophenyl)-4-methyl-5-(β -hydroxyethyl)-6-hydroxy pyrimidine :

Clean sodium metal (1.2 g) was added to 15 ml of absolute methanol. Then to this a mixture of 4-chlorobenzamidine hydrochloride (.48 g; 0.025 mole) and α -acetyl- β -butyrolactone (3.2 g; 0.025 mole) in 25 ml absolute methanol was added dropwise under stirring. The reaction mixture was stirred for 3 hours more and left overnight. It was then refluxed for 3 hours, filtered and the filtrate was concentrated under reduced pressure. The semisolid mass obtained after washing the residue with ether was dissolved in 50 ml of water and on acidification with acetic acid, a white precipitate was obtained. It was collected by filtration and washed with cold

water to get the title compound. It was recrystallised from ethyl alcohol.

Yield : 3.6 g (55.0%); m.p. 260-262°C.
 Anals. Calcd. for $C_{13}H_{13}ClN_2O_2$: C, 58.98;
 H, 4.91; N, 10.59%
 Found : C, 58.62; H, 4.65; N, 10.39%

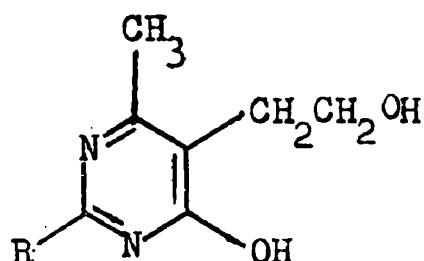
Biological Results and Discussion

In all, twenty-five pyrimidine derivatives, were synthesised (Table I) and only four representative members were screened for their *in vivo* antimalarial activity against *P. yoelii* by Rane's method (5). In this series, compounds Nos. 1, 2, and 3 (Table II) were active at 160 mg/kg. All these compounds were inactive at 40 mg/kg and also nontoxic upto the highest tried dose level of 160 mg/kg.

Acknowledgement

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TABLE I
2-Substituted-4-methyl-5-(β hydroxyethyl)-6-hydroxy
Pyrimidines



No.	R	M.p. °C ^a	Molecular formula. ^b
1.	4-ClC ₆ H ₄	260-62	C ₁₃ H ₁₃ ClN ₂ O ₂
2.	4-BrC ₆ H ₄	258-59	C ₁₃ H ₁₃ BrN ₂ O ₂
3.	4-FC ₆ H ₄	220-22	C ₁₃ H ₁₃ FN ₂ O ₂
4.	4-(n) PrOC ₆ H ₄	203-04	C ₁₆ H ₂₀ N ₂ O ₃
5.	3,4-(EtO) ₂ C ₆ H ₃	179-80	C ₁₇ H ₂₂ N ₂ O ₄
6.	3,4-EtO (MeO)C ₆ H ₃	176-77	C ₁₆ H ₂₀ N ₂ O ₄
7.	3,4-MeO (EtO)C ₆ H ₃	176-78	C ₁₆ H ₂₀ N ₂ O ₄
8.	3,4-MeO (n-PrO)C ₆ H ₃	179-80	C ₁₇ H ₂₂ N ₂ O ₄
9.	3,4-EtO (PhCH ₂ O)C ₆ H ₃	175-76	C ₂₂ H ₂₄ N ₂ O ₄
10.	3,4-MeO (PhCH ₂ O)C ₆ H ₃	216-18	C ₂₁ H ₂₂ N ₂ O ₄
11.	3,4-EtO (2'-ClPhCH ₂ O)C ₆ H ₃	208-10	C ₂₂ H ₂₃ ClN ₂ O ₄
12.	3,4-MeO (2'-ClPhCH ₂ O)C ₆ H ₃	191-92	C ₂₁ H ₂₁ ClN ₂ O ₄
13.	3,4,5-(MeO) ₃ C ₆ H ₂	175-76	C ₁₆ H ₂₀ N ₂ O ₅
14.	C ₆ H ₅ OCH ₂ -	172-73	C ₁₄ H ₁₆ N ₂ O ₃
15.	4-ClC ₆ H ₄ OCH ₂ -	198-20	C ₁₄ H ₁₅ ClN ₂ O ₃
16.	4-CH ₃ C ₆ H ₄ OCH ₂ -	192-93	C ₁₅ H ₁₈ N ₂ O ₃
17.	3-CH ₃ C ₆ H ₄ OCH ₂ -	166-68	C ₁₅ H ₁₈ N ₂ O ₃
18.	3-Pyridyl	212-14	C ₁₂ H ₁₃ N ₃ O ₂
19.	4-Pyridyl	242-44	C ₁₂ H ₁₃ N ₃ O ₂
20.	2-Chloro-4-pyridyl	240-42	C ₁₂ H ₁₂ ClN ₃ O ₂
21.	2-Phenoxy-4-pyridyl	204-05	C ₁₈ H ₁₇ N ₃ O ₃
22.	2-(4'-chlorophenoxy)-4-pyridyl	225-27	C ₁₈ H ₁₆ ClN ₃ O ₃
23.	2-(4'-Chloro-3'-methylphenoxy)-4-pyridyl	194-95	C ₁₉ H ₁₈ ClN ₃ O ₃
24.	2-(2',4'-Dichlorophenoxy)-4-pyridyl	205-08	C ₁₈ H ₁₅ Cl ₂ N ₃ O ₃
25.	2-(β Naphthoxy)-4-pyridyl	253-54	C ₂₂ H ₁₉ N ₃ O ₃

a = Melting points are capillary melting points and are uncorrected.

b = Analysed correctly for C, H and N within $\pm 0.4\%$.

TABLE II
Antimalarial activity against *P. yoelii*

r. No.*	MST, T or C** (Dosage in mg/kg)	
	40	160
1.	5.0	8.5 (A)
2.	4.3	11.0 (A)
3.	4.0	9.0 (A)
13.	4.0	4.0

* Numbers refer to those from Table-I, hydrochlorides of these compounds were screened.

** Δ MST is the mean survival time (days) of treated mice (MSTT) minus the mean survival time (days) of control mice (MSTC). Death prior to MSTC are toxic (T), mice which live more than 40 days are regarded as cured (C). Drugs which double the survival time of mice compared to control are considered active (A). In each run pyrimethamine was included as standard drug (curative dose = 4.5 mg/kg). (-) = Not tested.

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Book-Review

Membrane Transport in Biology Vol. I.
Ed. Tosteson D. C.
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It is only under rare circumstances that therapeutic agents are administered at the desired site of action. By and large drugs are administered into the body either by enteral or parenteral route with the fervent hope that they will achieve therapeutic levels at the site of action, and will not accumulate at other sites to produce side effects. While in the case of a number of drugs this distribution is merely hoped for in good faith, increasing number of studies are being carried out on the distribution of drugs in the body.

On the administration of a therapeutic agent at a site other than its site of action, the agent gets distributed in the various compartments of the body, in accordance to its solubility and permeability characteristics. Such a distribution requires the transfer of the agent in its native or modified form across a variety of biological barriers. While the barriers separating different compartment pairs are undoubtable of varied characteristics, all barriers share among each other a unifying factor — the cell wall. Thus the transfer of agents across biological barriers is basically the transfer across the cell wall. For this reason the cell wall or the unit biological membrane assumes importance when the transfer of agents or the transport of drugs is under consideration.

Springer Verlag has published a series of volumes on Membrane Transport in Biology, the first volume of which on concepts and models is under review. In general the volume is prepared with meticulous care, maintaining the high standard of the publications of the publishers.

For a reader being initiated into the field of drug and biological transport studies, the initiation necessarily begins with a study of the various transport mechanisms present in the cell wall. Sten-Knudsen and Stark in two chapters have dealt with passive transport mechanisms and carrier mediated transport exhaustively, concentrating more on the mathematical treatment of the processes. While a small chapter on other active processes would have been welcome, the two chapters would go a long way, by bringing together known data for the benefit of workers in the field. An entrant in the field of pharmacodynamics is however better off if, he spends a little time in going through other reviews on the topic of drug transport before taking up this volume.

Donald J. Hanahan, a pioneer in the field of lipid research, has contributed a chapter on the constitution of cell membranes. Most of the advance in the field of drug transport has been brought about only after the exact composition of the cell wall was revealed. The lipid bilayer wall contributes probably more to the peculiar properties of the cell wall, with particular reference to permeability than all other factors put together.

Hanahan's treatment of the topic leaves little to be desired.

Rudolf and Lefkowitz, in their chapter on Membrane receptors cyclic nucleotides and transport have made a detailed review of the literature on hormone receptor binding and permeability. The effect of cyclic nucleotides on cell wall permeability has been long since suggested and also amply demonstrated. So also the relation of cyclic nucleotides to hormonal stimulation of cells, Rudolf and Lefkowitz have presented a large amount of data adding weight to the second messenger hypothesis. Recently a good amount of evidence is surfacing which suggests that the cyclic nucleotides may not be as omnipotent as suggested before. The authors of this chapter have expressed a very balanced view stating 'recognizing the role of cyclic AMP has not provided any final answers,

since it is clearly not the final step. (of hormone receptor interaction).

Another interesting chapter is that on Black lipid films. The use of lipid film technology has revolutionized the study of membrane transport in-vitro. However, prior plunging into this chapter the reader is strongly advised to get himself acquainted with the basic techniques, as this chapter deals only with advanced experiments.

While greater emphasis has been laid on some chapters, this is not to say that other chapters are not interesting or important. The book is a valuable addition to a library catering the needs of pharmacologists, biologists, biochemists and such workers.

R. B. Ghooi
Scientific Secretary.

Proceedings of the Critic's Club

(August 1980 to April 1981)

Shri B. D. Sathe of the Department of Zoonosis addressed the members of the club on the 3rd Oct, 1980. He discussed the problem of schistosomiasis, a focus of which was reported in the village, Gimvi in Ratnagiri district of Maharashtra, as far back in 1952. In order to study the present status of the focus a study was undertaken by the Haffkine Institute. During the course of the study, 607 of the inhabitants of the village were examined, of which four were observed to excrete terminal spined schistosome ova in the urine. The skin test conducted with schistosome antigen revealed that about 20% of the population living in the endemic area reacted positively to the test while in non-endemic areas the positive responders ranged between 1.5 to 3.5%.

The department of Zoonosis which conducted the study also collected snails from and around the village. A large number of the collected snails were found to be infested with cercariae of two different types. From the data obtained the workers concluded that the focus in the village may not be of the human type.

The next meeting of the club was held on the 17th of October and was addressed by Shri S. R. Lokhande of the Department of Chemotherapy. Anthelmintic diseases are very common in our country as well as in other developing countries, and there is a crying need for better and cheaper drugs for the control of the disease. This institute has been involved in the synthesis and screening of new drugs for helminthic diseases,

since long. Shri Lokhande's talk was based on their attempts to prepare new thiazole derivatives for better activity. The worker reported the synthesis of thiazolyl phenylamino pyrido (3,2-d) thiazoles and discussed the structure activity relationships of the compounds.

Cell mediated immunity forms an important part in the immunological control of diseases. Shri Sharad Khedkar addressed the club on the 14th of November, describing his work on the involvement of the cell mediated immunity and humoral immunity in the response of mice to *Salmonella enteridis* infections. *S. enteridis* was demonstrated to evoke under certain conditions both the cell mediated and humoral response in different circumstances. While formalised organisms evoked a good humoral response, sub-lethal doses of live organisms evoked mainly cell mediated immunity. The implications of the data obtained were discussed by the worker with active participation of the audience in the discussion which followed.

The next meeting of the club, held on 28th of November was addressed by Shri R. B. Ghooi of the Director's Laboratory. The speaker described the use of an anaphylactoid agent known as Compound 48/80, to induce an oedematous reaction in the hind paw of mice, and inhibition of oedema genesis by different compounds. The worker suggested that this model is simpler than the existing models for antianaphylactic screening and as such could be used as a preliminary screening test for new compounds.

Dr. David Madden, the Deputy Chief Infectious diseases Branch and the Head of the Immunochemistry Section, of National Institutes of Health Bethesda, delivered a guest lecture at the next meeting of the club on 3rd of December. Dr. Madden has been one of the pioneers in the field of enzyme linked immunosorbant assays. In his laboratory he has succeeded in establishing a number of new assays for the diagnosis of viral diseases. His talk to the club included data obtained from his experiments, as well as general observations on the subject.

At the 12th December meeting of the Critic's Club, Shri Jagdish Deshpande of the Immunology Department spoke on the purification of mycobacterial antigens. The earlier work by the department had demonstrated that the component of mycobacterial antigens which elicits delayed hypersensitivity response was a lipoprotein. Shri Deshpande described his attempts to purify the lipoprotein using various chromatographic techniques. The data obtained suggests the molecular weight of the lipoprotein to be around 150,000 daltons.

Smt. A. C. Desai of the Department of Pharmacology addressed the Critic's Club at its meeting on the 19th of December. Smt. Desai has been working on the isolation, purification, and pharmacology of an anti-diabetic factor from *been* used by Indian physicians since Vedic times. The worker has isolated a glycoside from the plant which demonstrates hypoglycemic effect on normal and hyperglycemic laboratory animals. Further studies on the glycoside are in progress.

Dr. H. S. Ved of the Biochemistry Department spoke before the members of

the club on the 17th of January 1981. Dr. Ved has been studying the metabolism of lipids in the body and the effects of vitamins on the same. The studies conducted by him included the incorporation of radiolabelled acetate, phosphate and cholesterol in the lipid compartments of the liver. The data obtained reveals that dietary vitamin D is essential in maintaining optimal incorporation of lipid precursors.

On the 6th of February 1981, Dr. (Mrs.) C. H. Pandit of the Department of Virology spoke before the club on megalovirus infections. Cytomegaloviruses comprise a group of agents within the herpes virus family and have widespread distribution in human and other animals. In the past the diagnosis of cytomegalovirus infection was based on histological study of post-mortem tissues. Recently the study of exfoliative cytology of urine samples has made it possible to diagnose the disease within the life time of the patient. Dr. Pandit discussed the clinical features, laboratory diagnosis and their results with light microscopy.

Miss V. Saraswathy of the Bacteriology Department addressed the 20th February meeting of the club. The topic of her talk was plant growth substances elaborated by actinomycetes. In the recent years a lot of attention has been duly focussed on plant growth substances. Miss Saraswathy gave a detailed description of their work on Actinomycetes, which are found in abundance around the root zone of plants. Screening a large number of Actinomycetes species, the workers have detected plant growth substances in a few species.

R. B. Ghooi
Scientific Secretary.

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Contributions should be precise and concise, highlighting original observations and inferences. The type-script (T.S.) should be sent in duplicate, typed in double space on only one side of the sheet with ample margins. A brief summary not exceeding 200 words, should accompany the article. The title should be informative and specific, followed by author's name and address.

A short title in not more than forty letters should also be suggested. The text of the paper should be generally sub-divided into: Introduction, Materials and Methods, or Experimental, Results, Discussion, Acknowledgement and References.

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Results should be presented concisely in tables giving only important and relevant data and avoiding incorporation of elaborate observations.

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(i) Reuben, R. ; Carey, D.E. and Meyers, R. M. (1968) — Japanese encephalitis studies in Vellore, S. India, **Indian J. Med. Res;** **56**, 109-18.

(ii) Cruickshank, R. (1968) — Medical Microbiology, 11th Ed. 180-4, E and S Livingstone Ltd., Edinburgh, p. 28.

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