Original Article *Ind. J. Tub., 1992, 39, 21*

EFFECT OF ADMINISTRATION OF RIFAMPICIN ON THE ADRENOCORTICAL FUNCTION IN PATIENTS WITH PULMONARY TUBERCULOSIS

G. Raghupati Sarma[1], Chandra Immanuel[2], P.V. Krishnamurthy[3], Rani Balasuhramanian[3], Geetha Ramachandran[4] and R. Prabhakar[5]

(Original received on 14.2.91; Revised version received on 25.6.91; Accepted on 8.8.91)

Summary. **Adrenocortical function was studied on admission and during treatment in 57 newly-diagnosed patients with pulmonary tuberculosis, 16 of whom were treated with a daily regimen containing Rifampicin (R-7), 22 with a twice-weekly regimen containing the same drug (R-Z) and 19 with a daily regimen that did not contain Rifampicin (NR-7). In patients on daily treatment (R-7 and NR-7), there was a slight increase in plasma cortisol at 1 week followed by a decline; while the mean level at 4 weeks was similar to that on admission in the R-7 patients, that in the NR-7 patients was significantly lower ($P < 0.01$). No change was observed in the R-2 patients. A positive response to tetracosactrin was observed in the 7 R-7, 14 R-Z and 7 NR-7 patients on admission and in 6, 14 and 14, respectively at 4 weeks. The increase in the proportion of positive responders in the NH-7 patients was significant ($P = 0.05$). On admission, the diurnal rhythm of the release of cortisol, as assessed by changes in salivary cortisol, was disturbed in the patients with an evening rise in the cortisol levels; it had, however, reverted to a near-normal pattern after 2 months of treatment in all 3 groups of patients.**

Introduction

Tuberculosis is believed to be one of the important causes of adrenal insufficiency in man. Investigations undertaken recently in patients with pulmonary tuberculosis at Tuberculosis Research Centre, Madras, had shown that the plasma cortisol concentrations were high and nearly 50% of the patients had a negative response to tetracosactrin, suggesting a lack of adrenal reserve (or functional adrenocortical insufficiency) in these patients[1]. Further, the diurnal rhythm of cortisol release was disturbed in these patients, with an evening rise in cortisol levels; the pattern of changes observed in saliva was similar to that observed in plasma (unpublished findings), though the concentrations were much lower. Rifampicin is a known inducer of the hepatic microsomal enzyme system and has been shown to cause an enhanced clearance of endogenous cortisol[2] and also of exogenously administered corticosteroids[3,4,5,6]. The occurrence of acute adrenal crisis in patients on treatment with drug regimens containing Rifampicin has also been reported[7,8]. We, therefore, undertook an investigation to study the effect of two different rhythms of administration of Rifampicin (daily and twice-weekly) on the response to tetracosactrin (given on admission and after 4 weeks of treatment) and compared the findings with those observed in patients who received a daily regimen that did not contain the drug. The diurnal rhythm of the release of cortisol, as assessed by cortisol levels in saliva, was also examined on admission and after 2 months of treatment in all the 3 groups of patients. This report presents the findings.

Material and Methods

Patients and treatment regimens: Sputum smear-positive patients with pulmonary tuberculosis were randomly allocated to one of the following

1. Deputy Director; 2. Research officer; 3. Assistant Director; 4. Research Assistant; 5. Director, Tuberculosis Research Centre (Indian Council of Medical Research), Madras.

Correspondence : Dr. G. Raghupati Sarma, Deputy Director, Tuberculosis Research Centre, Chetput, Madras-600 031.

regimens (during the initial intensive phase of 2 months) :

R-7: Rifampicin 450 mg plus Ethambutol 800 mg plus Isoniazid 300 mg plus Pyrazinamide 1.5 g daily.

R-2: Rifampicin 450 mg plus Ethambutol 1.6 g plus isoniazid 600 mg plus Pyrazinamide 2.0 g twice-weekly.

NR-7: Same as R-7 above, but without Rifampicin.

None of the patients was diabetic or pregnant or on any form of steroid treatment at the time of the investigation.

Plasma cortisol and tetracosactrin stimulation test : Blood was collected between 08:00 and 08:30 hours on admission and at 1, 2 and 4 weeks after the start of treatment, and plasma cortisol determined. The tetracosactrin stimulation test was performed on admission and at 4 weeks. On both occasions, blood was collected for the determination of the basal plasma cortisol and at ½ hour and 1 hour after intramuscular administration of 0.25 mg (about 24 units) of tetracosactrin (kindly donated by Hindustan Ciba Geigy Ltd). Plasma was separated and stored at –20°C till assay.

The patients were classified as positive or negative responders to tetracosactrin, the criterion for a positive response being an increase in plasma cortisol of 200 nmol/l or more at ½ hour and 1 hour over the respective basal level[1].

Diurnal variation of cortisol level : This was studied from changes in salivary cortisol on admission and at 2 months after the start of treatment. Saliva was collected at 08:00 hour and then at 2-hourly intervals up to 20:00 hour. The saliva specimens were frozen (kept at -20°C) overnight. They were then thawed and centrifuged; the residue containing mucoproteins was discarded and the clear supernatant stored at -20°C till assay.

Plasma cortisol was determined by a standard RIA technique employing commercially available cortisol assay kit (Amersham International plc, England), and salivary cortisol was estimated according to a method, adapting these kits, standardized at our Centre (unpublished).

Student's t-test (paired and unpaired) was employed for testing the difference between the mean cortisol levels, the chi-square test with Yates' correction for continuity to test the differences between the proportions in the different treatment groups, and McNemar's test for testing the differences between the proportions within the same group at different time-points.

Results

A total of 61 patients (54 male, 7 female) was admitted to the study; of these, 17 were allocated to the R-7 regimen, 23 to the R-2 regimen and 21 to the NR-7 regimen. Four patients (1 R-7, 1 R-2, 2 NR-7) were excluded from the analysis due to culture negativity on admission, inadequate (< 80%) chemotherapy or failure to attend on one or more of the designated test-days. The analysis is therefore based on 57 patients (16 R-7,22 R-2, 19 NR-7). The culture grading for *M. tuberculosis* on admission was 3 + (confluent growth) in 35 patients, 2 + (> 100 colonies) in 19, 1 + (20-100 colonies) in 2 and less than 20 colonies in 1 patient.

Plasma cortisol levels : The mean basal plasma cortisol levels (08:00 hour) in the 3 groups, on admission and at 1, 2 and 4 weeks after the start of treatment are presented in Table 1 and Figure 1. The mean values were similar in the 3 groups on admission. In the R-7 patients, the mean plasma cortisol at 1 week was about 24% higher ($P = 0.06$) than on admission. Further treatment caused a decrease in the levels and the mean value at 4 weeks was about 14% less than that at 1 week, and similar to that on admission. In the NR-7 patients too, the mean value at 1 week was about 11% higher ($P = 0.06$) than that on admission and further treatment caused a decrease and the mean value at 4 weeks was about 21% less than that at 1 week ($P < 0.01$); the difference between the mean values on admission and at 4 weeks (12%) was also significant ($P < 0.01$). There was practically no change in the mean basal plasma cortisol values at different weeks in the R-2 patients.

At 1 and 2 weeks, only the mean value in the R-7 patients was significantly higher than that in the R-2 patients ($P = 0.01$ and 0.04, respectively), and at 4 weeks, only the mean value in the R-7

Table. 1. *Mean plasma cortisol levels on admission and up to 4 weeks of treatment with daily (R-7) and twice-weekly (R-2) regimens containing Rifampicin and a daily regimen without the drug (NR-7)*

Group	Mean ± standard deviation of plasma cortisol values (nmol/l)			
	On admission (0 week)	1 week	2 weeks	4 weeks
R-7 (n = 16)	471 ± 173	582 ± 140	550 ± 101	502 ± 130
R-2 (n=22)	449 ± 132	455 ± 153	448 ± 186	456 ± 138
NR-7 (n= 19)	466 ± 153	516 ± 126	481 ± 136	410 ± 142

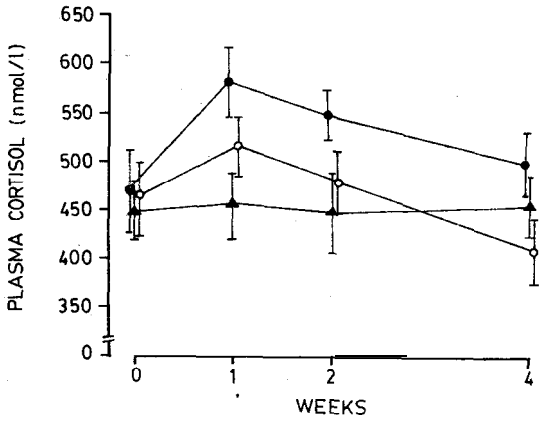

Fig. 1. Mean plasma cortisol levels (nmol/l) on admission (0 week) and at 1, 2 and 4 weeks after the start of treatment in R-7 (•—•), R-2 (||—||) and NR-7 patients (o—o). Vertical bars indicate the standard error of the mean.

patients was higher than that in the NR-7 patients (P = 0.05).

Response to tetracosactrin : The response of patients to tetracosactrin on admission and at 4 weeks after the start of treatment is given in Table 2. On admission, 7 of 16 R-7, 14 of 22 R-2 and 7 of 19 NR-7 patients had a positive response to tetracosactrin, the total number of patients with a positive response being 28 (49%) of 57 patients. The proportion of positive responders among the R-2 patients was higher than those in the other two groups, the differences not being significant. Among the R-7 patients, 4 were positive and 7 negative responders both on admission and at 4 weeks; 3 patients who had a positive response initially became negative responders and 2 who were negative responders initially had a positive response at 4 weeks. Among the R-2 patients, the classification did not alter in 14 patients; of the remaining 8 patients, 4 who had a positive response initially became negative responders and 4 who were negative initially had a positive response at 4 weeks. Among the NR-7 patients, the classification was the same on admission and at 4 weeks in 10 patients; 8 patients who were initially negative became positive responders, and 1 patient who was initially positive became a negative responder at the end of 4 weeks. The proportions of patients with a positive response to tetracosactrin at 4 weeks were 6 of 16 R-7,14 of 22 R-2 and 14 of 19 NR-7 patients. The increase in the proportion of positive responders at 4 weeks over that on admission was significant in the NR-7 patients (P = 0.05), and there was a suggestion that this proportion was higher than that in the R-7 patients at 4 weeks (P = 0.07).

The association between sputum culture grading for *M. tuberculosis* and plasma cortisol or positive response to tetracosactrin on admission in the 57 patients is presented in Table 3. The mean plasma cortisol value in patients with a 3 + grading was significantly higher than that in patients with a grading of 2 + or less (P = 0.01). The difference between the proportions of positive responders among patients with a grading of 3 + and 2 + or less was not significant. Sputum became negative for *M. tuberculosis* in 5 of 16 R-7, 3 of 22 R-2 and 5 of 19 NR-7 patients by 1 months. No significant associations were observed between the bacteriological status at 1 months and plasma cortisol or the proportion of positive responders to tetracosactrin at 4 weeks (data not presented).

Dismal variation of salivary cortisol : The mean salivary cortisol levels being similar in the R-7, R-2 and NR-7 patients on admission and at 2 months were, therefore, amalgamated for further analysis. The mean salivary cortisol levels at the different time-points during the 12-hour period of collection on admission and at 2 months, together

Table. 2. *Response to tetracosactrin on admission and after 4 weeks of treatment with daily (R-7) and twice-weekly (R-2) regimens of Rifampicin and a daily regimen not containing the drug (NR-7)*

Response to tetraco-sactrin on admission (0 week)	Response to tetracosactrin at 4 weeks								
	R-7			R-2			NR-7		
	Positive	Negative	Total	Positve	Negative	Total	Positive	Negative	Total
Positive	4	3	7	10	4	14	6	1	7
Negative	2	7	9	4	4	8	8	4	12
Total	6	10	16	14	8	22	14	5	19

Table. 3. *Mean plasma cortisol and number of patients with positive response to tetracosactrin according to sputum culture grading for M. tuberculosis on admission*

Culture grading	No. of patients	Mean plasma cortisol ± standard deviation (nmol/l)	Positive response to tetracosactrin No.	%
3+	35	504 ± 143	15	43
2+	19		12	
1+	2	393 ± 131	1	59
1-19 colonies	1		0	
	57	461 ± 149	28	49

with the distribution curve for health subjects from an earlier study are presented in Table 4 and Figure 2. On admission, there was a decline in salivary cortisol between 08:00 and 18:00 hours ($P < 0.001$) followed by an increase, and the mean value at 20:00 hour was significantly higher than that at 18:00 hour ($P = 0.03$). After 2 months of treatment, the mean cortisol levels at the different time-points were significantly lower than the corresponding values on admission ($P < 0.02$). Further, there was a decrease in the cortisol levels beyond 18:00 hour, and the mean value at 20:00 hour was significantly lower than those at 18:00 hour and 08:00 hour ($P < 0.001$ for both). The pattern of change in salivary cortisol in the patients at 2 months was fairly similar to that observed in the healthy subjects.

Table. 4. *Diurnal variation of salivary cortisol in healthy subjects, and on admission and at 2 months after the start of treatment in patients with pulmonary tuberculosis*

Group	Month	Mean ± standard deviation of salivary cortisol values (nmol/l) at the following hours (clock time)						
		08:OO	l0:00	12:00	14:00	16:00	18:00	20:00
TB patients (n=57)	0	18.2 ± 12.7	19.2 ± 14.6	15.8 ± 13.1	15.7 ± 8.6	11.1 ± 9.1	11.1 ± 10.9	13.2 ± 12.3
	2	13.9 ± 5.7	11.2 ± 6.3	10.6 ± 4.3	12.0 ± 5.7	7.1 ± 4.5	7.1 ± 4.3	5.4 ± 2.8
Healthy subjects (n=6)		8.1 ± 4.1	6.2 ± 3.4	8.5 ± 2.3	6.7 ± 2.6	5.0 ± 2.4	3.8 ± 1.8	3.7 ± 1.4

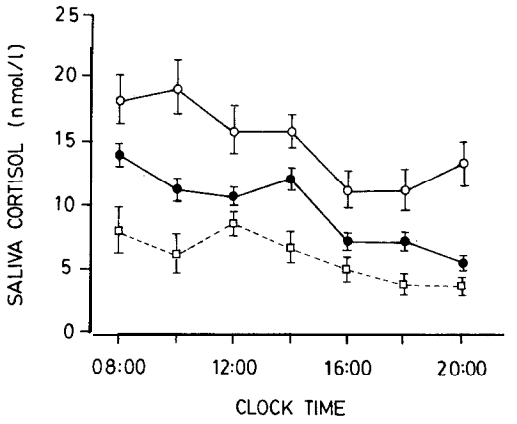

Fig. 2. Diurnal variation of salivary cortisol on admission (O—O) and at 2 months after the start of treatment (•—•) in patients with pulmonary tuberculosis, and in healthy subjects from an earlier study (•—•). Vertical bars indicate the standard error of the mean.

Discussion

Adrenocortical function appears to be compromised in patients with pulmonary tuberculosis. Despite high plasma levels of cortisol, nearly 50% of the patients are negative responders to tetracosactrin, suggesting a lack of adrenal reserve (or functional adrenocortical insufficiency). This inadequate response is not due to the high basal levels, as the association between the base-line levels and the increase at ½ hour and 1 hour was weak, the correlation co-efficients being -0.64 at ½ hour and -0.45 at 1 hour. Further, the diurnal rhythm of cortisol secretion is disturbed in these patients with an evening rise in cortisol levels. These findings are in close agreement with those reported earlier from this Centre[1].

Maximal induction of the hepatic microsomal enzyme system is known to occur after about a week of treatment with daily regimens containing Rifampicin[9,10] Hence, the systemic clearance of cortisol would also be expected to be high at this time. Our findings, however, show that there is a slight increase in plasma cortisol at 1 week in both the groups of patients who received daily treatment (R-7 and NR-7). Wilkins *et al*[8] have observed that there is a swelling of the adrenals soon after start of treatment, probably similar to the paradoxical enlargement of tuberculous lymph nodes and cerebral tuberculomas with the initiation of chemotherapy[11,12,13,14]. It is not known whether this enlargement of the adrenals is accompanied with an increase in the activity of the glands, and whether this is responsible for the initial increase in plasma cortisol observed in our study.

Plasma cortisol and adrenal function in tuberculous patients are governed by the stress due to the disease, and a possible damage to the adrenals due to the tuberculous process. Hence, treatment with effective anti-tuberculosis drugs should result in a decrease in the circulating plasma cortisol level and an increase in the proportion of patients with a positive response to tetracosactrin. Both these effects were seen in patients who did not receive Rifampicin (NR-7); however, in patients who received this drug (R-7 and R-2), the plasma cortisol levels at 4 weeks after the start of treatment were similar to those on admission and no increase was observed in the number of positive responders to tetracosactrin. On the contrary, 7 of 21 patients (in both the R-7 and R-2 groups), who had a positive response initially, became negative responders at 4 weeks as against 1 of 7 patients in the NR-7 group.

Response to tetracosactrin, on admission and during treatment in patients with tuberculosis has been studied by 3 other groups of investigators. Ellis and Tayoub[15] observed that 55% of 41 African Zulu patients had a sub-normal response to tetracosactrin and that the improvement in the response after 15 days of treatment was inferior in patients who received Rifampicin as one of the drugs than in those who did not. These authors did not report on plasma cortisol levels. In a recent report, Scott *et al*[16] observed a negative response to tetracosactrin in 4 of 18 British patients on admission. All the patients were treated with a daily regimen containing Rifampicin, Isoniazid and Ethambutol and these authors have reported that the expected decrease in plasma cortisol during treatment was not seen, an observation similar to our study; of the 4 patients with a sub-normal response, 1 died unexpectedly, 2 received steroids and 1 patient had a persistent sub-normal response to tetracosactrin. In a study by Barnes *et al*[17] in Melanesian patients, the proportion of those with a sub-normal response to tetracosactrin (8% of 90 patients with pulmonary, miliary or

extrapulmonary tuberculosis) was much less than that in our series, and this is most probably due to difference in the definition employed to classify the patients as positive or negative responders to tetracosactrin[1]. Further, they observed a significant decrease in plasma cortisol, 3-4 weeks after the start of treatment with a regimen containing daily Rifampicin in addition to Isoniazid, Pyrazinamide and Streptomycin, findings contrary to those reported in our study and by Scott *et al*[16]. The daily regimen employed by us (R-7) was similar to that used by Barnes *et al*[17], except that Streptomycin was replaced by Ethambutol, and it seems unlikely that this single change could be responsible for the different findings.

Findings in this paper suggest that Rifampicin, whether administered daily or twice-weekly exerts a deleterious effect on the adrenocortical function, in patients with pulmonary tuberculosis. The mechanism of action is not clear; the sustained high plasma cortisol concentrations, even up to 4 weeks in patients receiving Rifampicin, appear to go against a mechanism involving a more rapid systemic clearance of this hormone induced by the drug. Earlier investigations had shown that dexamethasone causes an appreciable suppression of cortisol levels in patients with pulmonary tuberculosis[1]. And this suggests that the HPA axis is intact in these patients. As such, a decrease in plasma cortisol caused by an increase in its clearance would trigger the release of ACTH by the pituitary resulting in an increase in the secretion of cortisol by the adrenal cortex. Whether formation of antibodies to Rifampicin or other immunological reactions such as the formation of immune complexes involving mycobacterial antigens precipitated by the rapid killing of the bacteria or the induction of auto-immune antibodies against adrenocortical cells are responsible, is a matter of conjecture. Several drugs such as procainamide, hydrallazine, methyldopa and pencillamine have been shown to induce auto-immune disorders such as systemic lupus erythematosis, myasthenia gravis, haemolysis and hepatitis[18] and Rifampicin has been implicated in evoking pemphigus, an auto-immune disease of the skin[19].

The diurnal rhythm of cortisol secretion was disturbed in the patients with an evening rise in cortisol levels before the start of treatment. The immune system is known to modulate adrenal production of glucocorticoids and cortisol, in turn, has been shown to regulate certain immune responses. Monokines, particularly interleukin-1, released by activated monocytes, are known to stimulate the hypothalamus, the pituitary and the adrenal cortex[20,21,22]. Stimulation of hypothalamus would not only cause an increase in the release of CRF, which would ultimately lead to an increase in the cortisol production, but also of certain other factors such as PGE_2 which cause a elevation in body-temperature[23]. An evening rise in body-temperature is well-established in patients with tuberculosis, but the association between the two phenomena pre-supposes a circadian rhythm in the release of monokines also. The existence of such a rhythm of several immune responses is now well-recognised. Thus, cytolytic activity of natural killer cells[24], mitogenic and antigenic responses[25,26,27,28], the counts of eosinophils and neutrophils[28] and of T and B cell populations of human lymphocytes[29,30] have been shown to exhibit a circadian rhythm. The maximal levels of T and B cells were attained at midnight followed by a depression during the day, and Abo *et al* have shown that variations in the cell counts were inversely related with plasma cortisol levels in healthy subjects.

The diurnal rhythm of cortisol secretion had reverted to a near-normal pattern after 2 months of treatment in all the 3 groups of patients. Response to tetracosactrin was not examined at this time and whether this also returns to normal in a majority of the patients with continued treatment with regimens containing Rifampicin remains to be investigated.

Acknowledgements

We are grateful to Hindustan Ciba Geigy Limited, Bombay, for their kind gift of synacthen. We wish to thank Mr. K. Jayasankar for technical assistance and the Clinic and Nursing Staff for organising the blood and saliva collections.

References

1. Raghupati Sarma G., Chandra Immanuel., Geetha Ramachandran., Krishnamurthy P.V., Kumaraswami V., Prabhakar R. Adrenocortical function in patients with pulmonary tuberculosis. Tubercle; 1990,71,277.

2. Ohnhaus E.E., Park B.K. Measurement of urinary 6-ß-hydroxycortisol excretion as an *in vivo* parameter in the clinical assessment of the microsomal enzyme-inducting capacity of antipyrine, phenobarbitone and Rifampicin. Eur. J. Clin. Pharmacol.; 1979, 15, 139.

3. Edwards O.M., Courtney-Evans R.J., Galley J.M., Hunter J., Tait A.D. Changes in cortisol metabolism following Rifampicin therapy. Lancet; 1974, ii, 549.

4. Buffington GA., Dominguez J.H., Piering W.F., Herbert L.A., Kauffman M., Lemann J. Interaction of Rifampicin and glucocorticoids. Adverse effect on renal allograft function. JAMA.; 1976, 236, 1958.

5. McAlister W.A.C., Thompson P;J., Al-Habet S.M., Rogers H.J. Adverse effects of Rifampicin on prednisolone disposition. Thorax; 1982, 37, 792.

6. McAlister W.A.C., Thompson P.J., Al-Habet S.M., Rogers H.J. Rifampicin reduces effectiveness and bio-availability of prednisolone. Br. Med. J.; 1983, 286, 923.

7. Elansary E.H., Earis J.E. Rifampicin and adrenal crisis. Br. Med. J.; 1983, 286, 1861.

8. Wilkins E.G.L., Hnizdo E., Cope A. Addisonian crisis induced by treatment with Rifampicin. Tubercle; 1989, 70, 69.

9. Acocella G., Pagani V., Marchetti M., Baroni G.C., Nicolis F.B. Kinetic studies on Rifampicin. I. Serum concentration analysis in subjects treated with different oral doses over a period of two weeks. Chemotherapy; 171, 16, 356.

10. Chandra Immanuel., Jayasankar K., Narayana A.S.L., Raghupati Sarma G. Self-induction of Rifampicin metabolism in man. Ind J. Med Res.; 1985, 82, 381.

11. Campbell I.A., Dyson A.J. Lymph node tuberculosis : A comparison of various methods of treatment. Tubercle; 1977, 58, 171.

12. Chambers S.T., Hendricks W.A., Record C., Rudge P., Smith H. Paradoxical expansions of intra-cranial tuberculomas during chemotherapy. Lancet; 1984, ii, 181.

13. Smith H. Paradoxical responses during the chemotherapy of tuberculosis. J. Infect.; 1987, 15, 1.

14. Jawahar M.S., Sivasubramanian S., Vijayan V.K., Ramakrishnan C.V., Paramasivan C.N., Selvakumar N., Paul S., Tripathy S.P., Prabhakar R. Short-course chemotherapy for tuberculous lymphadenitis in children. Br. Med. J.; 1990, 301, 359.

15. Ellis M.E., Tayoub F. Adrenal function in tuberculosis. Br. J. Dis. Chest; 1986, 80, 7.

16. Scott G.M., Murphy P.G., Gemidjioglu M.E. Predicting deterioration of treated tuberculosis by corticosteroid reserve and C-reactive protein. J. Infect; 1990, 21, 61.

17. Barnes D.J., Naraqi S., Temu P., Turtle J.R. Adrenal function in patients with active tuberculosis. Thorax; 1989, 44, 422.

18. Russel A.S. Drug-induced autoimmune disease. Clin. Immunol. Allergy; 1981, 1, 57.

19. Gange R.W., Rhodes E.L., Edwards C.O. Pemphigus induced by Rifampicin. Br. J. Dermatol; 1976, 95, 445.

20. Besedovsk H.O., del Ray A.E., Sorkin E. What do immune system and brain known about each other ? Immunol Today; 1983, 4, 342.

21. Woloski B.M.R.N.J., Smith E.M., Meyer W.J., Fuller G.M., Blalock J.E. Corticotrophin-releasing activity of monokines. Science; 1985, 235, 1035.

22. Whitcomb R.W., Linehan G.M., Wahl L.M., Khazek R.A. Monocytes stimulate cortisol production by cultured human adrenocortical cells. J. Clin. Endocrinol. Metab.; 1988, 66, 33.

23. Hamblin A.S. Lymphokines in haematopoiesis, cytotoxicity and inflammation. In Male D, Rickwood D, ed Lymphokines. Oxford : IRL Press 1988, 45-52.

24. Williams R.M., Kraus L.J., Dubey D.P., Yunis E.J., Halberg F. Circadian bioperiodicity in natural killer cell activity of human blood. Chronobiologia; 1979, 6, 172 (Abstract).

25. Tavadia H.B., Fleming K.A., Hume P.D., Simpson H.W. Circadian rhythmicity of human plasma cortisol and PHA-induced lymphocyte transformation. Clin. Exp. Immunol; 1975, 22, 190.

26. Kaplan M.S., Byers VS., Levin A.S., German D.F., Fudenberg H.H., Lecam L.N. Circardian rhythm of stimulated lymphocyte blastogenesis. J. Allergy. Clin. Immunol; 1976, 58, 180.

27. Leskila H., Molnar F.G., Soppi E. Biological rhythm of cell-mediated immunity in man. Clin. Exp. Immunol; 1976, 26, 253.

28. Carter J.B., Barr G.D., Levin A.S., Byers VS., Ponce B., Fudenberg H.H., German D.F. Standardization of tissue culture conditions for spontaneous thymidine-2-^{14}C incorporation by unstimulated normal human peripheral lymphocytes : circadian rhythm of DNA synthesis. J. Allergy. Clin. Immunol; 1975, 56, 191.

29. Abo T., Kawate T., Itoh K., Kumagai K. Studies on the bioperiodicity of the Immune response. I. Circadian rhythms of human T, B and K cell traffic in peripheral blood. J. Immunol; 1981, 126, 1360.

30. Miyawaki T., Taga K., Nagaoki T., Seki H., Suzuki Y., Taniguchi N. Circadian changes of T lymphocyte subsets in human peripheral blood. Clin. Exp. Immunol; 1984, 55, 618.