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CIRCADIAN RHYTHMICITY OF HUMAN PLASMA CORTISOL AND PHA-INDUCED LYMPHOCYTE TRANSFORMATION

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SUMMARY

In two experiments, lymphocytes from six healthy subjects were sampled every 4 hr, counted, and cultured with PHA. Plasma cortisol in the same blood samples was measured and showed an expected circadian variation in level. A circadian rhythm in lymphocyte transformability to PHA was found to coincide directly with the cortisol level but varied inversely with lymphocyte numbers. The relationship between cortisol and lymphocyte transformability could be fortuitous or might indicate a role for cortisol in controlling lymphocyte function *in vivo*.

INTRODUCTION

There are several reports concerned with corticoid-mediated effects on the human lymphoid/immune system, including depression of blood lymphocyte numbers, decreased T-cell rosette formation, and thymo-lymphatic involution (Caffey & Silbey, 1960; Fauci & Dale, 1974; Gordon, 1955; Yu *et al.*, 1974; Selye, 1936). Plasma cortisol has been shown by Bliss *et al.* (1953) to have a circadian rhythm and therefore there may be a similar periodicity in some lymphoid parameters. We have presented evidence of circadian rhythmicity in human lymphocyte numbers and transformation induced by phytohaemagglutinin (PHA), pokeweed mitogen, and tuberculin-purified protein derivative (Simpson *et al.*, 1973; Tavadia *et al.*, 1975). Our observation of a rhythm in PHA transformation has since been confirmed, in extent and timing, by L. Sackett and E. Haus of St Paul (personal communication, 1974). We now present plasma cortisol measurements made during our study and as expected this variable exhibited a circadian rhythm. The remarkably coincident rhythms in PHA transformation and cortisol suggest a cause and effect relationship.

MATERIALS AND METHODS

Two studies, 1 month apart, were carried out on the same six male caucasian medical students (ages 19–22). Throughout the 36-hr observation span they were confined to the hospital. During the day (08.00–23.00 hr) only light sedentary activity was allowed. At night (23.00–08.00 hr) they slept in single rooms with total

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'black out'. Any consistent bias due to differences in diet was avoided by use of a milk diet (1 pint every 4 hr)—and nothing else by mouth.

Ante-cubital blood was obtained by venepuncture every 4 hr. Aliquots of 4 ml were added to standard EDTA vials for total white cell count and blood films; 10 ml was heparinized for transformation studies. Lymphocytes were separated on a Ficoll–Isopaque gradient and the separated plasma frozen for cortisol studies. Triplicate 1 ml cultures containing 1×10^6 lymphocytes in Hams F10 medium with 20% pooled AB plasma were incubated for 72 hr with PHA (reagent grade, Wellcome laboratories, 0·02 ml/culture). [^{3}H]-thymidine was added to the culture 8 hr before harvesting, which was carried out by filtration on to glass fibre filter paper (Whatman GF/A) and the residue counted on a Nuclear Chicago MK 1 liquid scintillation system.

All materials used were obtained from one batch. The setting up of transformation cultures was commenced immediately and completed within 2 hr of venepuncture. To minimize the effect of technical bias and confirm reproducibility, the data presented are the pool of two separate studies on the same subjects. Each graph point therefore represents 12 (i.e. 6×2) actual observations and, in the case of PHA transformations, 36 (i.e. $6 \times 2 \times 3$) since each test was done in triplicate.

RESULTS

As Fig. 1 shows, there is a clear-cut circadian rhythm in the number of circulating lymphocytes with a total swing of 35%, which varies inversely with the plasma cortisol level. These findings agree with those of other workers (Bartter, Delea & Halberg, 1962; Halberg *et al.*, 1973). It is also seen in Fig. 1 that the PHA transformation rhythm coincides with the plasma cortisol rhythm, both being low at 00.00 hours and high at 08.00 hours. Non-overlapping standard errors in the unconverted data indicate that the rhythm in all three is significant.

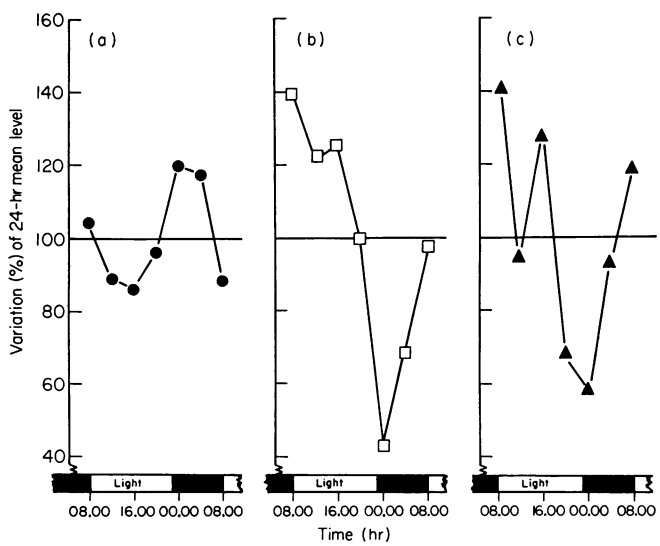

FIG. 1. The temporal relationship between the number of venous blood lymphocytes, their transformability with PHA and plasma cortisol. (a) Total lymphocyte count (mean 3019/mm^3); (b) PHA transformation (mean 81×10^3 d/min); (c) plasma cortisol (mean 6·6 μg/100 ml).

TABLE 1. Two duplicated studies 6 weeks apart (Y ± s.e.m.) from six male students (aged 19–22) on exclusive diet of 1 pint milk every 4 hr

		Time (hr)						
	Study	08.00	12.00	16.00	20.00	00.00	04.00	08.00
Total lymphocyte count (per mm^3)	A	3156 ±491	2652 ±308	2335 ±71	3022 ±164	3600 ±587	3414 ±172	2523 ±173
	B	3046 ±290	2698 ±198	2880 ±145	2750 ±248	3623 ±398	3726 ±347	2861 ±197
	A+B	3096 ±259	2675 ±175	2607 ±113	2886 ±147	3612 ±333	3570 ±191	2692 ±135
PHA transformation ($\times 10^3$ d/min)	A	149·6 ±41·7	156·9 ±15·1	66·8 ±5·3	72·0 ±10·2	51·1 ±5·3	62·9 ±5·4	111·3 ±24·3
	B	92·6 ±27·5	39·6 ±9·2	144·0 ±9·3	80·6 ±20·6	20·9 ±4·7	41·8 ±8·0	52·7 ±7·5
	A+B	123·7 ±26·3	98·3 ±19·6	66·9 ±5·4	76·7 ±11·7	36·0 ±5·6	52·4 ±5·6	81·9 ±15·0
Plasma cortisol (μg/100 ml)	A	10·0 ±1·2	7·7 ±1·6	11·5 ±1·5	6·7 ±2·4	6·5 ±1·9	8·6 ±1·6	7·0 ±1·8
	B	8·7 ±0·8	4·5 ±0·3	5·4 ±1·1	2·4 ±0·8	1·1 ±1·2	3·8 ±1·2	7·8 ±1·2
	A+B	9·3 ±0·7	6·2 ±1·0	8·4 ±1·3	4·5 ±1·3	3·8 ±1·2	6·2 ±1·2	7·8 ±1·2

DISCUSSION

The latter of these findings suggests that blood cortisol level may be exerting a direct positive effect on the potential of lymphocytes to transform in response to mitogen. Many studies (Fauci & Dale, 1974; Yu *et al.*, 1974; Roitt *et al.*, 1969; Nowell, 1961; Heilman, Gambrill & Leichner, 1973; Tormey, Fudenberg & Kamin, 1967) have shown that the artificial increase of steroid level *in vivo* or *in vitro* causes a decrease, rather than an increase, in amount of lymphocyte transformation. These studies are not, however, comparable to ours since either non-physiological levels of steroid were used, or when physiologically low levels were used the steroid was left in contact with the lymphocytes throughout the transformation test and may have produced increased fragility in the blast cells (Heilman & Leichner, 1971).

Another interpretation of these findings could be that although lymphocyte numbers are increased at a certain time during each day, the number of functional or operative lymphocytes remains the same at any given time. This would result in a reduced proportion of operative lymphocytes in the million cells taken for culture at times of peak lymphocyte numbers. Since the PHA-responsive lymphocyte is the T cell, an attempt is currently being made to measure percentage of T cells in the human lymphocyte population during 24 hr using spontaneous sheep cell rosettes as a marker for T cells.

Thus, the striking temporal association between the peripheral blood circadian rhythm of cortisol and PHA transformation, which we have demonstrated, may be a direct effect, although it is possible that the two parameters may be independently controlled by a third

factor. Our results have implications for interpretation of PHA lymphocyte transformation studies particularly those in which there is no environmental control or where the time (circadian stage) of venepuncture is regarded as unimportant. They may also be relevant when studying the effects of cortisol on immune responses.

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