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20 ABSTRACT (Continue on reverse side if necessary and identify by block number) Studies accomplished under this contract include both independent studies at the Harbor-UCLA Medical Center, Torrance, CA 90509, and collaborative studies of Navy personnel conducted with the Naval Health Research Center, San Diego, CA 92138. The focus of these studies was on the relationship between saliva and plasma steroid hormones, under basal conditions, suppression with dexamethasone, and simulated field conditions of exercise. → ccc /continued		

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The results demonstrate a very good correspondence between saliva and serum cortisol, such that saliva cortisol measures can be substituted for blood drawing for cortisol, as a non-invasive means of measuring levels of this stress-related hormone. Under conditions of physical exercise, saliva and serum cortisol levels did not change.

Results with testosterone suggest that saliva testosterone also reflects serum testosterone fairly closely, although the relationship is not as good as that for cortisol. Physical exercise did result in an approximately 20% increase in both plasma and saliva testosterone concentrations.

Finally, the correlation between saliva and serum melatonin was examined. While melatonin is measurable in saliva, it appears to correlate very poorly with serum melatonin, thus not being a useful reflection of serum melatonin. Work continues on the possibility of measuring a melatonin metabolite in saliva, which might more accurately reflect circulating melatonin levels in serum or plasma.



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FINAL REPORT

ONR CONTRACT N00014-81-K-0561

STRESS BIOCHEMISTRY: NON-INVASIVE MEASUREMENT
TECHNIQUES IN MILITARY SUBJECTS

OCTOBER 1980 TO JUNE 1983

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INTRODUCTION

Stress biochemistry has been an important area of medical research in the U.S. Navy for at least 25 years. Men and women under stressful field circumstances may suffer physiological changes resulting both in decrements in job performance and in clinical illness, so that the ability to measure physiologic changes before actual detrimental effects occur becomes very important. Part of the research performed by the Navy over the last two decades has been directed toward the elucidation of changes in several components of blood and urine in Navy subjects under stressful field conditions, including underwater demolition team trainees, submariners, Naval aviators making aircraft carrier landings, company commanders, and Navy and Marine recruits. These studies, in which the investigators on this proposal have participated (Rubin, 1974; Rubin and Rahe, 1974), have shown that many biochemical changes are related to quantitative changes in the perceived stress that the subjects are undergoing; examples include serum uric acid, cholesterol, cortisol, testosterone, and certain enzymes.

It has been possible to measure circulating concentrations of these hormones, enzymes, and metabolites in blood, following the advent of sensitive and specific radioimmunoassay techniques. Much stress-biochemical research has shown that blood concentrations of these measures are more reliable than urinary measures, which often are non-specific because of the inclusion of related metabolites as well as the parent compound itself (e.g. cortisol and testosterone). Therefore, stress-biochemical research in recent years has focused primarily on relevant measures in blood, which necessitates at least one, and frequently several, venipunctures to obtain samples. Because many hormones are secreted episodically, several blood samples must be taken over a relatively short period of time in order to accurately measure integrated hormone concentrations.

Military personnel in actual field circumstances often are undergoing considerable psychological and physical stress at a time when they are required to perform intricate and demanding tasks, so that research with invasive techniques such as venipuncture is not always feasible. Indeed, the venipuncture itself may be stressful to the individual and is known to change blood concentrations of several hormones, including the stress-responsive hormones cortisol and prolactin. In order to circumvent this problem, we have begun measuring steroid hormone concentrations in saliva as a non-invasive reflection of steroid hormone concentrations in serum or plasma. Steroid hormones are secreted into saliva by ultrafiltration, and several of them (cortisol, testosterone, progesterone) have been shown to closely reflect serum hormone concentrations (Riad-Fahmy et al, 1982). Many steroid hormones in blood circulate in both free and protein-bound forms, and it is the free fraction which is presumed to be physiologically active. Thus, saliva steroid hormone concentrations should reflect the free, physiologically relevant plasma concentrations and thus might actually provide more information than the measurement of total plasma hormone concentrations. For example, 24 hour saliva cortisol concentrations show a marked circadian variation, paralleling blood cortisol concentrations, and are similar to free cortisol concentrations in serum, which are 1% to 10% of the total serum cortisol concentration (Riad-Fahmy et al, 1982; see below).

WORK ACCOMPLISHED

We have measured saliva and serum cortisol concentrations in nine normal male volunteers who were injected intravenously with either saline or dexamethasone (0.5 mg) at 0800 hr, with blood and saliva samples collected at hourly intervals for the next 15 hours. Dexamethasone is a powerful synthetic glucocorticoid, which under normal circumstances suppresses the endogenous secretion of ACTH and cortisol for about 24 hours. Figures 1A and 1B show the results of this study. On the saline

injection day, the normal diurnal decline of cortisol was observed in both serum and saliva. Both the across-subjects and within-subjects correlations between serum and saliva cortisol concentrations were high ($r > 0.8$). However, the ratio of saliva to serum cortisol was somewhat variable across subjects and also changed within subjects depending upon the time of day. These differences in cortisol ratios may have been due to differences in free (unbound) cortisol concentrations in serum. Measurement of free cortisol concentrations in serum and comparison of these values to saliva cortisol concentrations currently are being done.

In an attempt to determine the threshold for cortisol suppression by dexamethasone, and the faithfulness of saliva cortisol measures to reflect this threshold, we undertook a dose-response study of nocturnal low-dose dexamethasone administration in normal volunteers. Figure 2 shows the results of this investigation. Three normal men and three normal women were given various doses of dexamethasone orally, in random order, once a week, for five consecutive weeks. Each subject took dexamethasone at home at 2300 hr and reported to the laboratory at 0700 hr the following morning, at which time single blood and saliva samples were obtained. Dose-response curves were obtained in all six subjects, with all subjects showing suppressed serum cortisol concentrations (< 50 ng/ml) at a dexamethasone dose of 0.5 mg. Some of the subjects also had suppressed serum cortisol after 0.375 mg. The cortisol response to dexamethasone did not appear to be related to body weight, since REP (the heaviest subject) showed reasonably good cortisol suppression after dexamethasone 0.25 and 0.375 mg, whereas LL (the lightest subject) only suppressed after dexamethasone 0.5 mg. Of importance is that the same pattern of cortisol suppression was observed in saliva. Again, these data indicate that saliva cortisol determinations are useful in assessing adrenal cortical activity.

Further evidence for the utility of saliva cortisol determinations comes from our studies of endogenously depressed patients and their normal matched control subjects. Some patients with endogenous depression show an early cortisol escape from dexamethasone suppression. We measured saliva and serum cortisol concentrations in 11 endogenously depressed patients and nine control subjects before and 8, 16, and 24 hours after dexamethasone administration (1.0 mg, orally) at 2300 hr. Figure 3 illustrates the results of this study. Six of the 20 subjects had post-dexamethasone serum cortisol concentrations > 50 ng/ml and thus were considered escapers from dexamethasone suppression. These six subjects also had saliva cortisol concentrations which were significantly elevated compared to those of the suppressors. Here, again, measurement of saliva cortisol is shown to be useful as a non-invasive technique for assessing adrenal cortical function.

In order to extend these studies to work circumstances, plasma and saliva cortisol and testosterone concentrations were measured in samples taken twice at rest and twice during exercise in normal men to determine whether physical activity affected either hormone. Fifteen male military and civilian personnel working for the U.S. Navy in San Diego completed a 30 minute run on a treadmill at 75% of maximum oxygen consumption capacity. They walked at 3 mph at 0% grade for four minutes to warm up. The speed then was increased by 0.5 mph each minute until a comfortable pace between 7 and 8 mph was reached. The grade then was increased 3% per minute until the subjects voluntarily stopped running. Continuous oxygen consumption was measured by spirometry, and maximum oxygen consumption capacity was determined for each subject. The next day the subjects returned to the laboratory at the same time of day for the 30 minute test run. Each ran on the treadmill at a speed calculated to represent 75% of his maximum oxygen consumption capacity. The first saliva and blood samples were taken prior to the run. The subjects then warmed up at 6 mph for five minutes and then ran for 15 minutes, at which time saliva and blood samples again were taken. The subjects then completed the last 15 minutes of the run, at which time they gave the final saliva and blood samples.

Table 1 presents the results of this study. Cortisol concentrations did not change significantly, but testosterone levels did. Plasma and saliva cortisol concentrations were highly correlated ($r > 0.8$), whereas plasma and saliva testosterone correlations were consistently lower ($r < 0.7$). Thus, moderately heavy exercise did not affect plasma and saliva cortisol, or the relationship between them, whereas changes in mean testosterone concentrations during exercise were statistically significant and similar in pattern for both plasma and saliva. The rather low correlation between plasma and saliva testosterone could have been due to the relatively restricted range of testosterone values in these subjects, compared to those of other studies (Wang et al, 1981).

Finally, we have been investigating the feasibility of measuring melatonin in saliva. Melatonin is a pineal hormone which has a clear circadian rhythm, highest concentrations in plasma occurring in the middle hours of the night during sleep (Wetterberg, 1983). We measured saliva and serum cortisol and melatonin concentrations in 10 normal male volunteers during one night of complete sleep deprivation, between 1900 and 0600 hr. Figure 4 shows the results of this study. The normal late-night nadir of both serum and saliva cortisol, and their increase beginning at 0230 hr, is evident. The normal nocturnal increase of serum melatonin also is evident, but this increase unfortunately is not reflected in saliva melatonin concentrations. Thus, for this pineal hormone, in contrast to the steroid hormones, saliva measures do not appear to be an acceptable substitute for serum measures. Perhaps a derivative or metabolite of melatonin could be measured in saliva and might more accurately reflect changes in serum melatonin concentrations. We are investigating this possibility, but it depends upon the availability of appropriate measurement techniques for melatonin metabolites.

PUBLICATIONS TO DATE UNDER THIS CONTRACT

1. Poland RE, Rubin RT (1982) Saliva cortisol levels following dexamethasone administration in endogenously depressed patients. *Life Sciences* 30:177-181.
2. Vickers RR, Hodgdon JA, Bennett EL, Poland RE, Rubin RT (1982) Salivary and plasma testosterone and cortisol during moderately heavy exercise. Technical Report 82-5, Naval Health Research Center, San Diego, California.

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pituitary-adrenal axis in health, endocrine and psychiatric conditions.
Psychoneuroendocrinology 8:75-80.

TABLE I

Plasma and Saliva Concentrations of Cortisol and Testosterone:

		<u>Mean Levels and Intercorrelations</u>							
		<u>Base-</u>	<u>Pre-ex</u>	<u>15 Min.</u>	<u>30 Min.</u>	<u>Average</u>	<u>F value</u>	<u>Significance</u>	
		<u>line</u>							
Cortisol	Plasma	Mean ^a	84.40	94.93	91.87	95.27	91.62	2.17	.145
		S.D.	22.72	32.44	29.77	33.81	29.55		
	Saliva	Mean ^a	1.89	2.31	2.12	2.47	2.20	1.84	.194
		S.D.	.88	1.54	1.06	1.60	1.30		
	Plasma/Saliva Correlation		.82***	.87***	.86***	.95***	.88***		
Testosterone	Plasma	Mean ^a	5.37	5.54	6.58 [#]	6.73 [#]	6.05	5.40	.014
		S.D.	1.74	1.58	2.60	2.21	2.11		
	Saliva	Mean ^a	.079	.085	.105	.115 [#]	.096	4.19	.030
		S.D.	.033	.019	.030	.036	.033		
	Plasma Saliva Correlation		.65**	.24	.48*	.37	.51*		

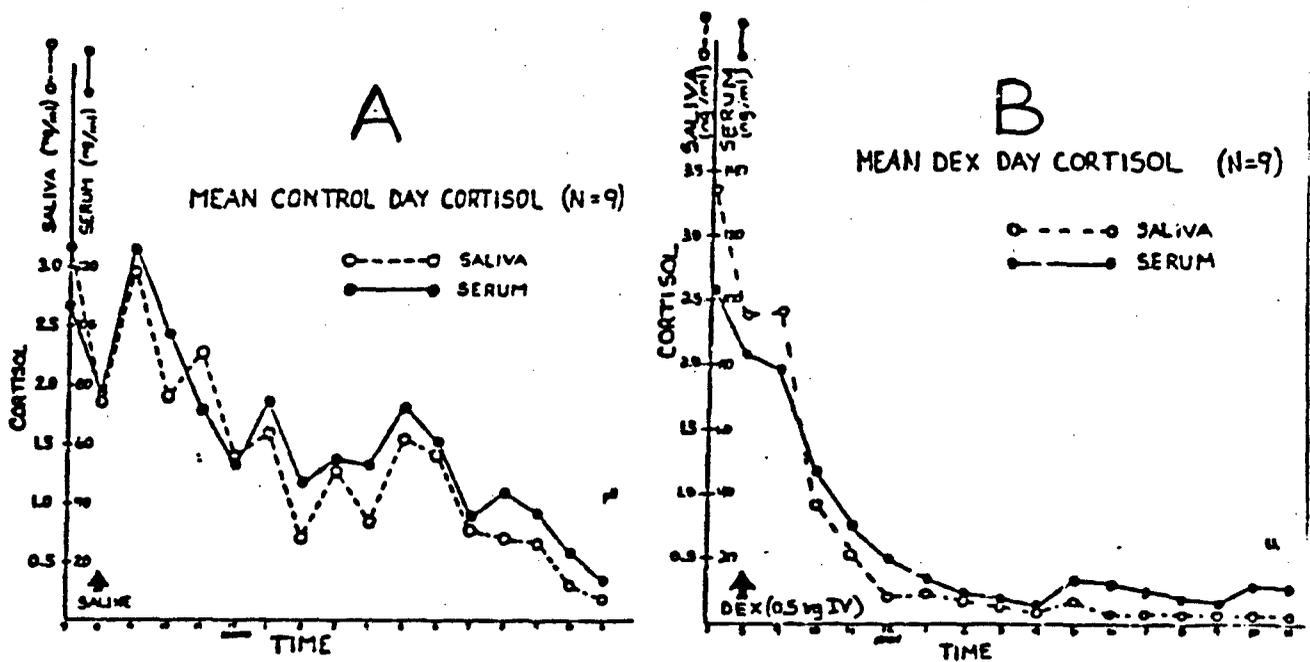
* p<.05, one-tailed

** p<.01, one tailed

*** p<.001, one-tailed

[#] Significantly different from baseline and pre-exercise values.

^a All concentrations reported in ng/ml



Figures 1A and 1B. Effect of saline (A) and dexamethasone 0.5 mg, intravenously (B), administered at 0800 hours, on serum and saliva cortisol concentrations for the next 15 hours in nine normal young adult men.

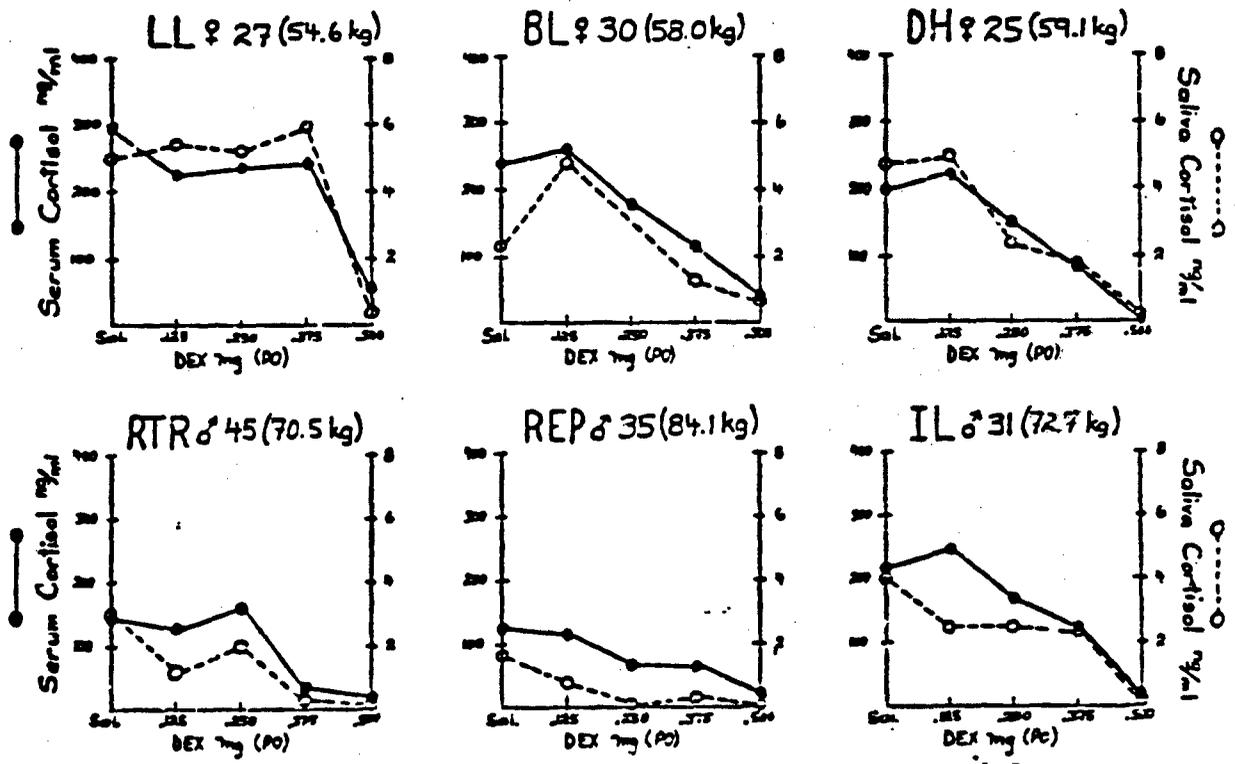


Figure 2. Serum and saliva cortisol concentrations at 0700 hours following oral administration of different doses of dexamethasone at 2300 hours the previous night in six normal volunteer subjects. Also shown are the sex, age, and body weight of each of the subjects.

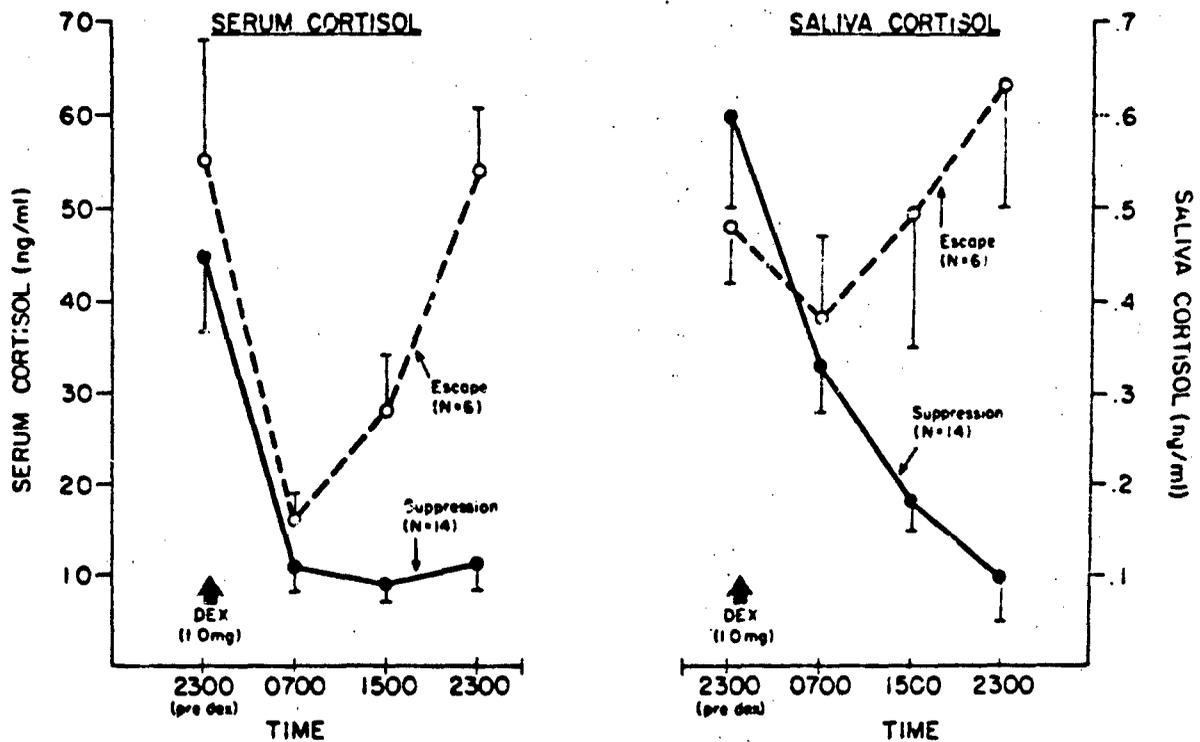


Figure 3. Mean (\pm SEM) pre-dexamethasone (2300 hours) and post-dexamethasone (0700, 1500, and 2300 hours) serum and saliva cortisol concentrations in 11 endogenously depressed patients and nine control subjects. Serum and saliva cortisol levels were significantly elevated in the six cortisol "escapers" compared to the 14 suppressors at both 1500 hours ($p < 0.01$) and 2300 hours ($p < 0.001$).

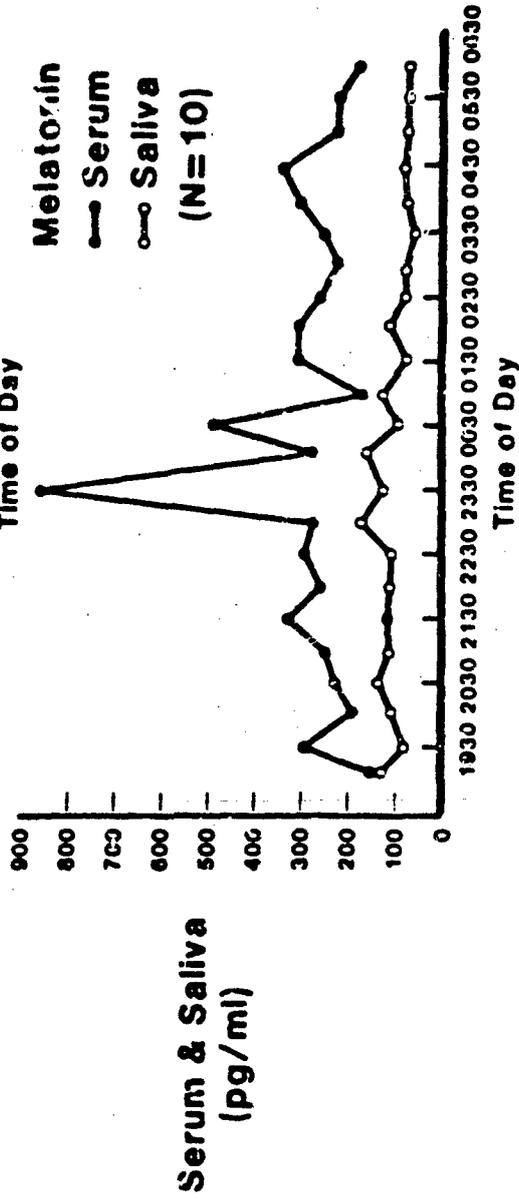
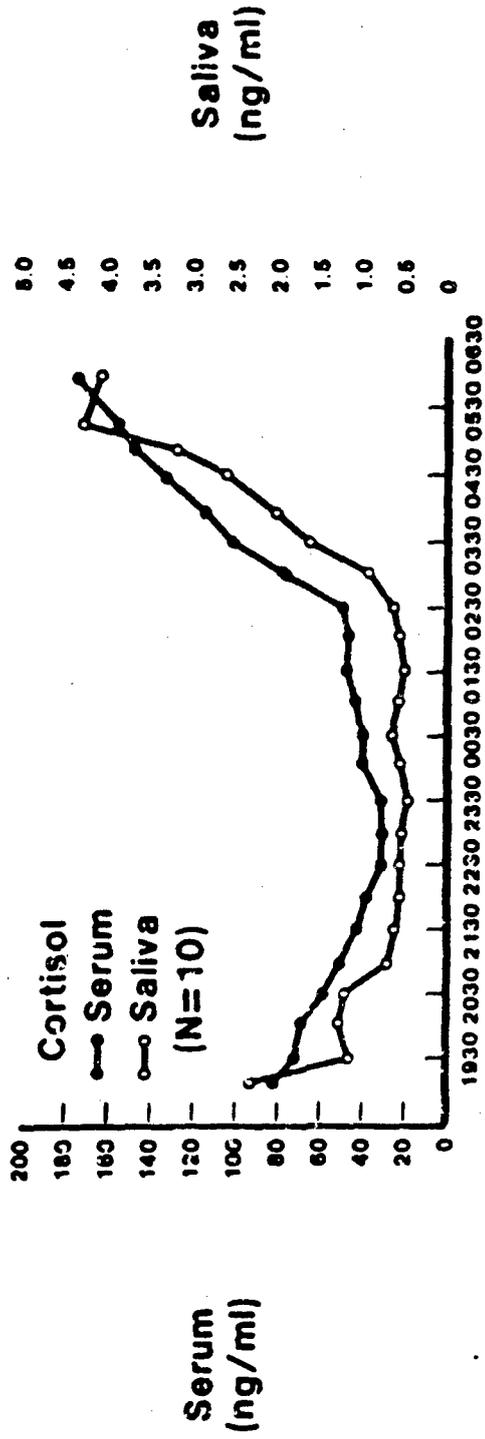


Figure 4. Serum and saliva cortisol and melatonin concentrations in 10 normal young adult men during one night of sleep deprivation.