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RHYTHMS AND TESTOSTERONE METABOLISM*

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SUMMARY

We have reviewed our data and others with regard to the diurnal variation in plasma testosterone and some of the factors which may affect the metabolism of the steroid and thus alter its plasma concentration.

INTRODUCTION

In 1965, using a double isotope derivative technique, we described a diurnal rhythm in the plasma levels of testosterone in young men [1]. The initial study (Fig. 1) was carried out with pooled specimens from six subjects. The highest level was found in the morning and the lowest in the late evening. The female subjects showed no significant diurnal variation. Subsequently (Fig. 2), plasma specimens were obtained from two male subjects, aged 25 and 30 years, at 90 min intervals for a period of 24 h (sixteen specimens) [2]. In addition, samples of blood were obtained from these subjects at 4 hour intervals (six specimens) during the same period of time. Similarly timed specimens were obtained from two normal young women. The plasma from the 90 minute collections were pooled and analyzed in duplicate for testosterone. The value obtained was considered a close approximation of the integrated daily plasma concentration of testosterone and this was compared to the individual values found in the 4 h specimens. A distinct diurnal variation in the plasma levels of testosterone is seen in both men. The highest concentration of the steroid was at 9 a.m. in each subject. The lowest level was reached at 2 a.m. in subject A.H. and 10 p.m. in subject D.L. The maximum fall in plasma levels of the androgen was 48% and 27% of the 9 a.m. values respectively. The plasma testosterone level found in the pool of sixteen specimens from each subject was 22% lower than the respective plasma testosterone levels seen at 9 a.m. Thus, the use of a morning plasma level of testosterone for the calculation of an integrated daily plasma production rate of the steroid in men introduces a significant error (although it does provide an "instantaneous" plasma production rate ($MCR^{T}_{1/day} \cdot PC^{T}_{\mu g/l}$). The magnitude of this error is proportional to the extent of the diurnal variation of plasma testosterone. An approximation of the integrated daily plasma level of testosterone can be obtained by using a pool of the 9 a.m., 5 p.m. and 10 p.m. specimens.

The presence of a diurnal rhythm in plasma testosterone has been confirmed in the human by many investigators [3–9] although not by all [10–12]. Moreover, diurnal cyclicity of plasma testosterone has been reported for the male rhesus monkey [13] although the phases of the rhythm were the reverse of that reported for the human. A diurnal variation in plasma testosterone was also found in the male

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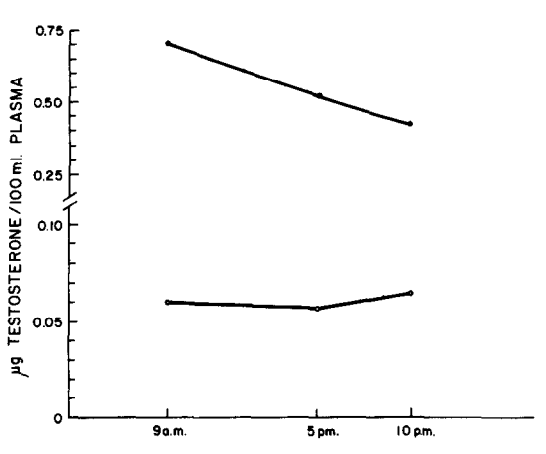

Fig. 1. Concentration of testosterone in pooled plasma specimens obtained at 9 a.m., 5 p.m. and 10 p.m. from six normal male (top) and six normal female (bottom) volunteers (from Southren *et al.*[1]).

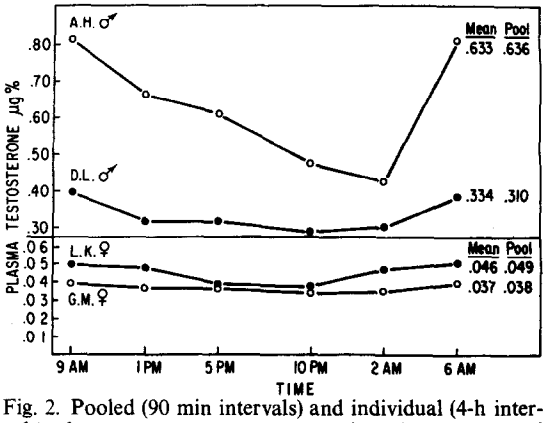

Fig. 2. Pooled (90 min intervals) and individual (4-h intervals) plasma testosterone concentrations in two normal young men (top) and two normal young women (bottom) during a 24-h period (from Southren *et al.* [2]).

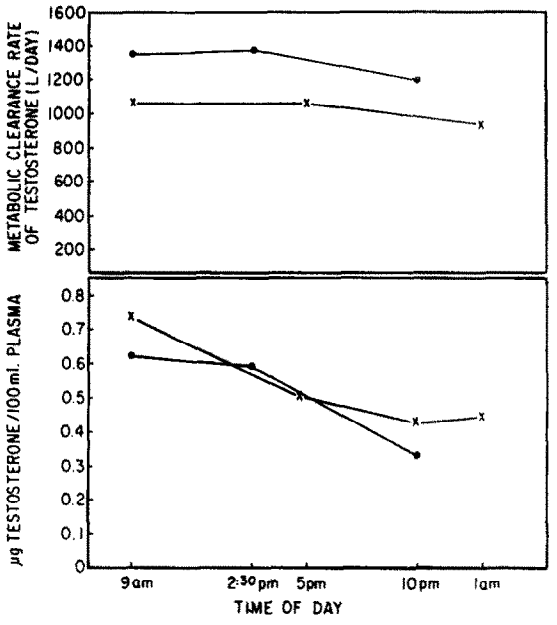

Fig. 3. Metabolic clearance rates and plasma concentrations of testosterone in two normal male subjects at various times during the day (from Southren *et al.* [2]).

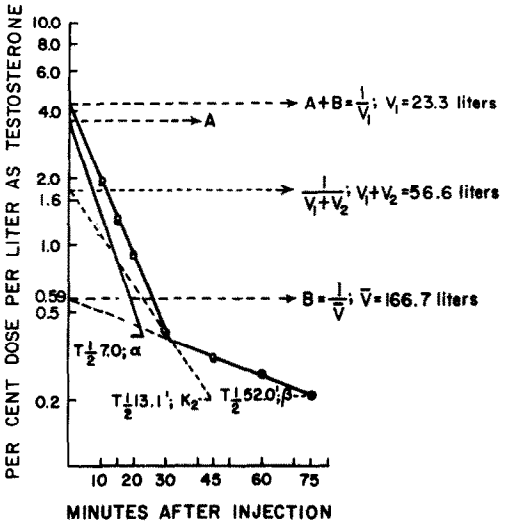

Fig. 5. Disappearance of 1,2-^{3}H-testosterone in plasma of normal male volunteer. Line connecting open circles represents line of best fit as determined by method of least squares. Closed circles are measured values (from Southren *et al.*[1]).

laboratory rat [14]. There is some evidence suggesting that the secretion of testosterone is not only diurnal but episodic in nature in men [15–17] and may be related to REM sleep [18]. Similarly, serum LH has been shown to be secreted episodically throughout the day although a positive correlation between serum LH and testosterone could not be generally demonstrated [15, 17] [19–24]. Nankin and Troen[19] however, showed that the repetitive abrupt elevations of plasma LH were highest in the early morning hours and Ruben *et al.*[25] found that the plasma LH values were higher during REM periods when compared to other stages of sleep. This nocturnally augmented secretion of LH was found by Boyer *et al.*[26] to be most prominent in puberal children of both sexes. Judd *et al.*[27] subsequently showed that in adolescent boys the sleep related LH increase stimulates a nocturnal rise in plasma testosterone and that this increase could account for some of the early puberal changes in males. More recently, it has been suggested that the mechanism for the diurnal variation of plasma testosterone is related to the presence of a diurnal variation in the responsiveness of the leydig cell to the gonadotropins [27]. This awaits confirmation. In addition to the already described diurnal variation and episodic secretion of testosterone, evidence for longer term periodicity in the endocrine function of the testes has been presented using such parameters as the urinary excretion of testoster-

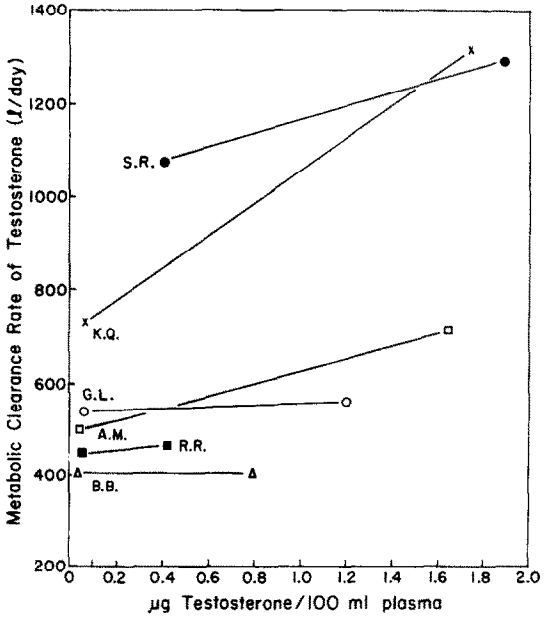

Fig. 4. Effect of acute increases in the plasma concentration of testosterone on the MCR^T in a normal man (S.R.), normal menstruating women (G.L., R.R., A.M. and K.Q.) and in a bilaterally adrenalectomized castrated woman (B.B.) (from Southren *et al.*[33]).

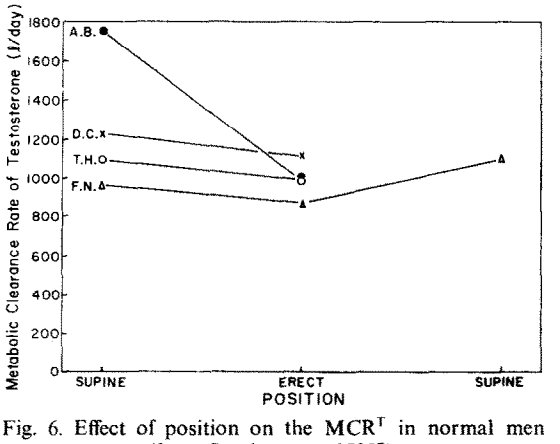

Fig. 6. Effect of position on the MCR^T in normal men (from Southren *et al.*[33]).

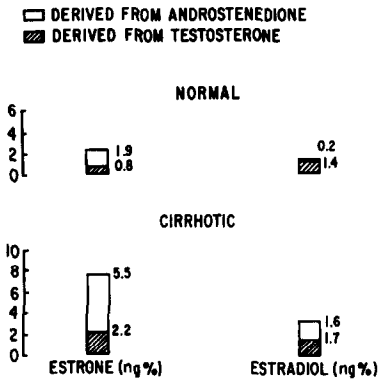

Fig. 7. The contribution (CR_{BB}^{Pre}·PC^{Pre}) of plasma testosterone and androstenedione to estrone and estradiol in normal and cirrhotic men [40].

one [29], beard growth [30, 31] and semen changes [31, 32].

The demonstrations of a diurnal variation in the concentration of plasma testosterone raised the question as to whether this phenomenon represented an inherent characteristic similar to that seen in many other biological systems or was related to an alteration in the peripheral metabolism of the steroid. In order to answer this question, the plasma levels of testosterone and the overall rate of metabolism of the steroid as measured by the metabolic clearance rate (MCR^T) were determined at intervals during a 24 h period under basal conditions in the supine position in two normal subjects using a constant infusion procedure [2] (Fig. 3).

As can be seen, there was no significant variation found in the values for the MCR's of the steroid during the 24-h period, while the concomitantly measured plasma concentrations of the hormone decreased sharply. This showed that the observed diurnal variation in plasma testosterone was due to a decrease in the production rate of the steroid rather than to an alteration in its metabolism. Moreover, acute increases in the plasma concentration of testosterone (Fig. 4) were not associated with any significant change in the plasma clearance of the steroid

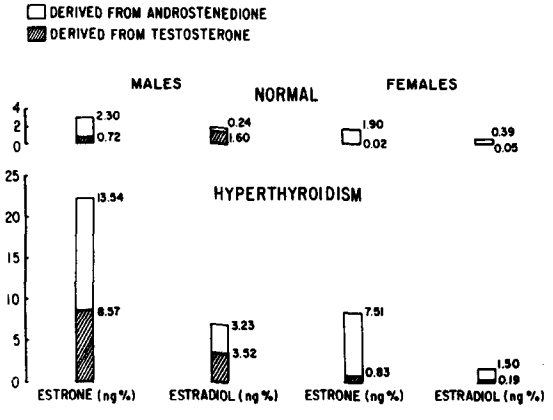

Fig. 8. The contribution (CR_{BB}^{Pre}·PC^{Pre}) of plasma testosterone and androstenedione to estrone and estradiol in normals and in hyperthyroidism (from Southren *et al.*[41]).

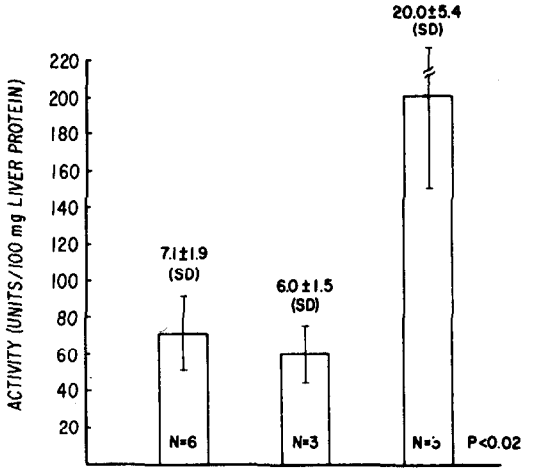

Fig. 9. The effect of medroxyprogesterone acetate on human hepatic testosterone A-ring reductase activity [43].

unless the values attained equalled or exceeded 1·6 μg% [33]. This critical level may be related to the capacity of the specific binding protein for testosterone. Thus, it is unlikely that the episodic secretion of testosterone, within the physiological range, is associated with an alteration in the rate of metabolic degradation but is more likely a reflection of an increased secretion of the steroid. Measurement of the half-life of testosterone in plasma based upon the rate of decline following a burst of secretion of the steroid denotes clearance of the hormone from the inner or plasma compartment. In this regard, we found the half-life of testosterone in this compartment (Fig. 5) to be approximately 13 min following a single injection of ^{3}H-testosterone [1]. This value is close to the 11 minutes recently reported by Vermuelen *et al.*[34] using the descending limb following an episodic secretory burst of the steroid. It should be noted, however, that measurement of the overall metabolism of testosterone requires a multicompartment analysis similar to that shown in Fig. 5.

Recent studies have focused on the episodic nature of the secretory processes of hormones. However, changes in the circulatory level of a hormone could result also from alterations in its metabolism or volume of distribution. Our laboratory has focused on those factors controlling the metabolic removal

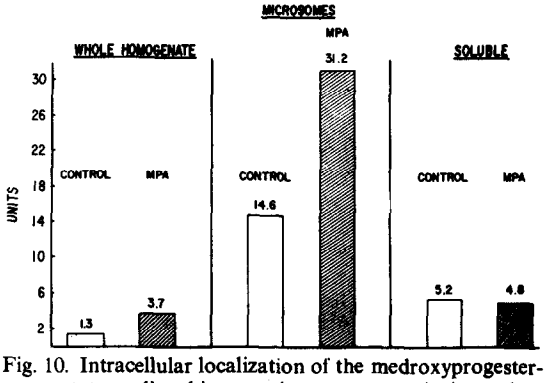

Fig. 10. Intracellular localization of the medroxyprogesterone acetate mediated increase in testosterone A-ring reductase activity [45].

of hormones from the blood. These changes would most likely be pertinent to the long term fluctuations in hormone levels rather than to the acute changes of the steroid.

For steroids, such as testosterone which have both hepatic and extrahepatic metabolism, the MCR is the sum of the products of each individual tissue extraction and its local blood flow. The tissue extraction which can be determined by appropriate techniques, is a function of both local diffusion processes and the level of activity of steroid metabolizing enzymes at each respective tissue site.

Assuming the upright position (Fig. 6) may produce a fall in the MCR^T in some but not all individuals. The fall is most likely due to an acute decrease in hepatic blood flow [33]. These findings are comparable to that previously reported by Lipsett *et al.*[5]. In instances in which a significant fall occurs in the MCR^T, it may alter the plasma level of the steroid. Cirrhosis of the liver is also associated with a decreased MCR^T which is, in part at least, related to a decreased hepatic blood flow [35].

The MCR^T, which is a measure of its irreversible tissue uptake, is a function of that fraction of the circulating steroid which is not bound to the specific high affinity plasma binding proteins. Cirrhosis of the liver [35], administration of estrogens [36] and hyperthyroidism [37, 38] are associated with an increased concentration of the high affinity testosterone binding proteins in plasma with resultant decreased MCR of the steroid. In both cirrhosis [39] and hyperthyroidism [40] the increased binding may be related to increased circulating estrogens due to an increased conversion of androgens to estrogens. The latter are depicted in Figs. 7 and 8. The instantaneous contribution of testosterone and androstenedione to estrone and estradiol were measured in cirrhotic men during infusion with the respective labelled precursors. It can be seen that androstenedione and testosterone contribute significantly to the circulating estrogens. We have estimated that 60% of the estradiol in male cirrhotics is derived from peripheral conversion from androgen precursors. Similarly, increased peripheral conversion of androgens to estrogens are seen in spontaneous hyperthyroidism. In the males, this increased conversion accounts for most of the circulating estradiol.

The metabolic degradation of testosterone is further influenced by tissue steroid metabolizing enzymes particularly the rate limiting 5α-steroid A-ring reductases. The activity of these enzymes are increased by various drugs such as progestogens [41]

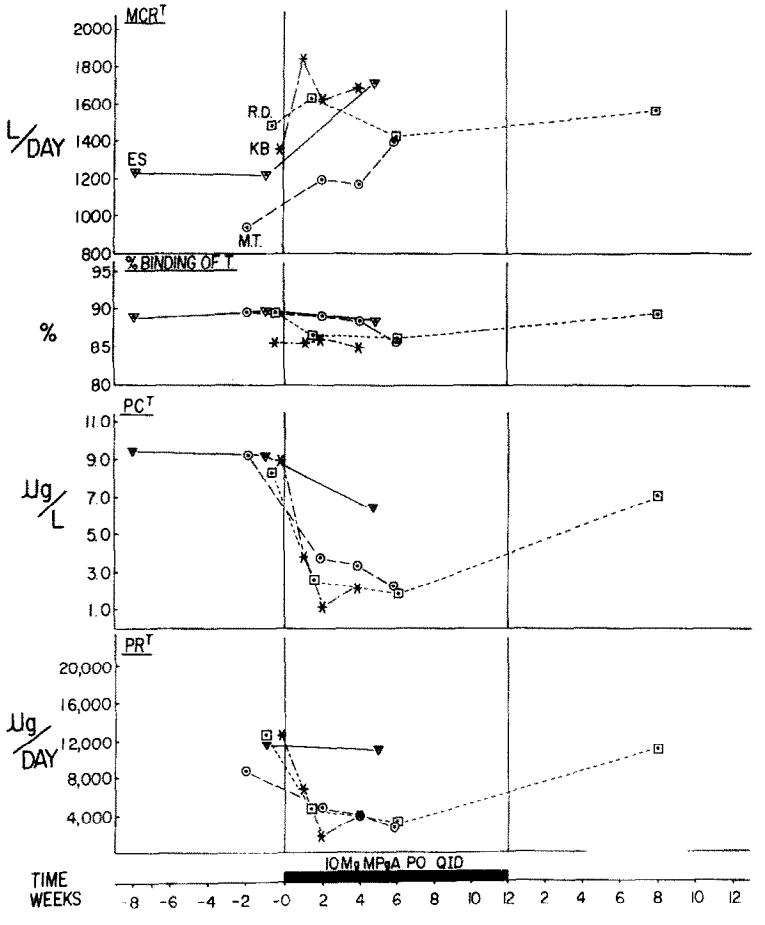

Fig. 11. Effect of medroxyprogesterone acetate on the metabolic clearance rate of testosterone, the percentage binding of testosterone in plasma, the plasma concentration and plasma production rates of the steroid in four normal men (from Gordon *et al.*[44]).

[42] and alcohol [43]. Figure 9 shows the stimulating effect of the progestational agent medroxyprogesterone acetate on the activity of human hepatic 5α-steroid A-ring reductase obtained from liver biopsy material and analyzed by a semi-micro technique [42]. The increased enzyme activity was found to reside in the hepatic microsomes [44] (Fig. 10). Further studies in our laboratory have established that the increased enzyme activity is due to an inductive effect of the drug as a result of synthesis of new enzymic protein [44]. This conclusion was based on the similarity of the K_m of the induced and uninduced enzyme and the inhibitory effect of actinomycin on the induction. Administration of the progestational agent to male subjects (Fig. 11) produces a marked fall in the plasma level of testosterone. This is due to two factors. The first is the sharp rise in the MCR^T secondary to an increase in the activity of hepatic 5α-steroid A-ring reductase. The second is related to the gonadotropin suppressing effect of the drug. Alcohol also increases the activity of 5α-steroid A-ring reductase and may alter the MCR^T [43]. This study was carried out in normal volunteers. Each subject was studied before and after daily iso-caloric ingestion of 300 ml absolute alcohol (diluted to 20% in orange juice) over a period of three to four weeks. The mean increase in reductase activity was 120%.

Thus, many factors must be considered when evaluating plasma levels of testosterone over a prolonged period of time.

REFERENCES

1. Southren A. L., Tochimoto S., Carmody N. C. and Isurugi K.: *J. clin. Endocr. Metab.* **25** (1965) 1441–1450.
2. Southren A. L., Gordon G. G., Tochimoto S., Pinzon G., Lane D. R. and Stypulkowski W.: *J. clin. Endocr. Metab.* **27** (1967) 686–694.
3. Dray F., Reinberg A. and Sebaoun J.: *C. r. hebd. Séanc. Acad. Sci. Paris* **261** (1965) 573–576.
4. Resko J. A. and Eik-Nes K. B.: *J. clin. Endocr. Metab.* **26** (1966) 573–576.
5. Lipsett M. B., Wilson H., Kirschner M. A., Korenman S. G., Fishman L. M., Sarfaty G. A. and Bardin C. W.: *Recent Progr. Horm. Res.* **22** (1966) 245–281.
6. Crafts R., Llerena A., Guevara A., Tobotsky J. and Lloyd C. W.: *Steroids* **12** (1968) 151–163.
7. Nieschlag E. and Ismail A. A. A.: *Klin. Wochenschr* **48** (1970) 53–54.
8. Faiman C. and Winter J. S. D.: *J. clin. Endocr. Metab.* **33** (1971) 186–192.
9. Okamoto M., Setaishi C., Nakagawa K., Horiuchi Y., Moriya K. and Itoh S.: *J. Clin. Endocr. Metab.* **32** (1971) 846–851.
10. Hudson B., Coghlan J. P., Dulmanis A. and Wintour M.: Excerpta Med. Intr. Cong. Ser. **83** (1964) 1127–1134.
11. Kirchner M. A., Lipsett M. B. and Collins D. R.: *J. clin. Invest.* **44** (1965) 657–665.
12. Kobayoshi T., Lobotsky J. and Lloyd C. W.: *J. clin. Endocr. Metab.* **26** (1966) 610–614.
13. Plant T. M. and Michael R. P.: *Acta endocr., Copenh.*, Suppl. **155** (1971) 69.
14. Kinson G. A. and Lui Chung-ching: *Horm. Metab. Res.* **5** (1973) 233–234.
15. Naftolin F., Judd H. L. and Yen S. C.: *J. clin. Endocr. Metab.* **36** (1973) 285–288.
16. West C. D., Mahajan D. K., Chavre V. J., Nabors C. J. and Tyler F. H.: *J. clin. Endocr. Metab.* **36** (1973) 1230–1236.
17. Murray M. A. F. and Corker C. S.: *J. Endocr.* **56** (1973) 157–158.
18. Evans J. I., MacLean A. W., Ismail A. A. A. and Love D.: *Nature, Lond.* **229** (1971) 261–262.
19. Nankin H. R. and Troen P.: *J. clin. Endocr. Metab.* **33** (1971) 558–560.
20. Naftolin F. S., Yen S. S. C. and Tsai C. C.: *Nature* **236** (1972) 92–93.
21. Boyar R. M., Perlow M., Hellman L., Kapen S. and Weitzman E.: *J. clin. Endocr. Metab.* **35** (1972) 73–81.
22. Krieger D. T., Ossowski R., Fogel M. and Allen W.: *J. clin. Endocr. Metab.* **35** (1972) 619–623.
23. Boyar R. M., Perlow M., Kapen S., Lefkowitz G., Weitzman E. and Hellman L.: *J. clin. Endocr. Metab.* **36** (1973) 561–567.
24. deLacerda L., Kowarski A., Johanson A. J., Athanosiori R. and Migeon C. J.: *J. clin. Endocr. Metab.* **37** (1973) 366–371.
25. Ruben R. T., Kales A., Adler R., Fagan T. and Odell W.: *Science* **175** (1972) 196–198.
26. Boyar R., Finkelstein J., Roffwarg H., Kapen S., Weitzman E. and Hellman L.: *N. Engl. J. Med.* **287** (1972) 582–585.
27. Judd H. L., Parker D. C., Siler T. M. and Yen S. S. C.: *J. clin. Endocr. Metab.* **38** (1974) 710–713.
28. Judd H. L., Parker D. C., Rakoff J. S. and Yen S. S. C.: Proceeding of the 55th Meeting of the Endocrine Society, 1973, Abstract No. 103.
29. Ismail A. A. A. and Harkness R. A.: *Acta endocr., Copenh.* **56** (1967) 469–480.
30. Anon: *Nature* **226** (1970) 869–871.
31. Kihlstrom M.: *Life Sci.* Part II **10** (1971) 321–324.
32. Doggett V. C. and Keillers R. K.: *Anat. Rec.* **142** (1962) 227.
33. Southren A. L., Gordon G. G. and Tochimotos S.: *J. clin. Endocr. Metab.* **28** (1968) 1105–1112.
34. Vermeulen A., Verdonck L. and Comhaire F.: In *Biorhythms and Human Reproduction*, (Edited by M. Ferrin, F. Halberg, K. M. Richart and R. L. VandeWiele). John Wiley & Sons, New York, 1974, 427–445.
35. Southren A. L., Gordon G. G., Olivo J., Rafii F. and Rosenthal W. S.: *Metabolism* **22** (1973) 695–702.
36. Tochimoto S., Olivo J., Southren A. L. and Gordon G. G.: *Proc. Soc. exp. Biol. Med.* **134** (1970) 700–702.
37. Crepy O., Dray D. and Seboaun J.: *C.r. hebd. Séanc. Acad. Sci., Paris* **264** (1967) 2561.
38. Gordon G. G., Southren A. L., Tochimoto S., Rand J. J. and Olivo J.: *J. clin. Endocr. Metab.* **29** (1969) 164–170.
39. Gordon G. G., Olivo J., Rafii F. and Southren A. L.: *J. clin. Endocr. Metab.*, in press.
40. Southren A. L., Olivo J., Gordon G. G., Vittek J., Brener J. and Rafii F.: *J. clin. Endocr. Metab.* **38** (1974) 207–214.
41. Gordon G. G., Southren A. L., Tochimoto S., Olivo J., Altman K., Rand J. and Lemberger L.: *J. clin. Endocr. Metab.* **30** (1970) 449–456.
42. Gordon G. G., Altman K., Southren A. L. and Olivo J.: *J. clin. Endocr. Metab.* **32** (1971) 457–461.
43. Gordon G. G., Altman K. and Southren A. L.: Proceeding of the 56th Annual Meeting of the Endocrine Society, 1974, Abstract No. 372.
44. Altman K., Gordon G. G., Southren A. L., Vittek J. and Wilker S.: *Endocrinology* **90** (1972) 1252–1260.