4 G.M. Vaughan, R.J. Reiter, T.M. Siler-Khodr, J.W. Sackman, J.P. Allen, M.K. Vaughan, W.L. McGuire, L.Y. Johnson and P. Starr, Experientia *34*, 1378 (1978).
5 R.A. Hoffman and R.J. Reiter, Anat. Rec. *153*, 19 (1965).
6 R.J. Reiter, D.C. Klein and R.J. Donofrio, J. Reprod. Fert. *19*, 563 (1969).
7 J.P. Allen, D.M. Cook, J.W. Kendall and R. McGilvra, J. clin. Endocr. Metab. *37*, 230 (1973).
8 S. Furuyama, D. Mayes and C. Nugent, Steroids 16, 415 (1970).
9 J.L. Bruning and B.L. Kintz, Computational Handbook of Statistics. Scott, Foresman and Co., Glenview 1968.
10 M. Motta, O. Schiaffini, F. Piva and L. Martini, in: The Pineal Gland, p.279. Ed. G. Wolstenholme and J. Knight. Churchill Livingstone, Edinburgh and London 1971.
11 M.K. Vaughan, G.M. Vaughan, R.J. Reiter and B. Benson, Neuroendocrinology *10*, 139 (1972).
12 J. Dunn, M. Bennett and R. Peppler, Proc. Soc. exp. Biol. Med. *140*, 755 (1972).
13 D.T. Krieger, Endocrinology. *93*, 1077 (1973).
14 T.F. Ogle and J.I. Kitay, Endocrinology. *98*, 20 (1976).
15 J.J. Jacobs, Am. J. Anat. *139*, 437 (1974).
16 K. Takahashi, K. Inoue and Y. Takahashi, Endocr. jap. *23*, 417 (1976).
17 I. Nir, U. Schmidt, N. Hirschmann and F.G. Sulman, Life Sci. *10*, 317 (1971).

DOCA administration increases renal phospholipase activity in the rat[1]

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Summary. The phospholipase activity of renal tissue has been evaluated in controls and in DOCA treated rats. DOCA treated animal showed a higher than normal enzyme activity. Since a phospholipase is the key step in prostaglandin biosynthesis, it is suggested that the increased prostaglandin release promoted by mineraloactive steroids is mediated by an activation of this key enzyme.

Recent studies strongly suggest that interactions capable of modifying renal function occur between the kidney kallikrein-kinin and prostaglandin systems, as well as with mineraloactive steroids. In fact, the kallikrein-kinin system has been shown to influence the release of renal prostaglandins[3,4]. On the other hand, increased urinary kallikrein excretion has been observed in man with primary aldosteronism[5], and in animals receiving sodium retaining steroids[6]. Since the interaction between the kallikrein-kinin system and prostaglandins seems to take place through an activation of a phospholipase A_2[7], we have evaluated the phospholipase activity of renal tissue in DOCA-treated rats.

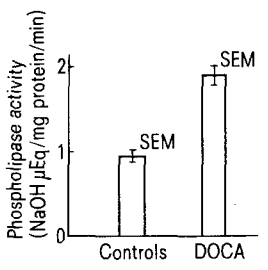
Experimental. The study was carried out in 16 male Sprague-Dawley albino rats weighing 230–280 g, which were divided into 2 groups. Group 1 consisted of 8 rats which were injected with 25 mg DOCA acetate in 1 ml sesame oil every 5 days for 20 days, and were fed a standard laboratory diet with free access to 0.9% NaCl as drinking fluid. Group 2 consisted of 8 control animals which were injected every 5 days with sesame oil alone, and were fed the same diet, but had free access to tap water as drinking fluid.

Blood pressure was measured employing a photometric cell and a proximal cuff on the tail of the conscious prewarmed animal. At the end of the study the animals were killed under ether anesthesia. The kidneys were excised, washed with 0.25 M sucrose until the venous effluent was clear, decapsulated, minced with a razor blade, and homogenized in a Potter-Thomas homogenizer using 6 ml sucrose. The homogenates were then centrifuged at 3000 rpm for 10 min in a Sorvall centrifuge at 4 °C, the precipitate was discarded, and the clear supernatant was employed for the determination of protein concentration and phospholipase activity. Protein concentration was determined according to Lowry et al.[8], employing bovine serum albumin as a standard. The assay of phospholipase activity was carried out as previously described[9] and expressed as μeq. NaOH/min/mg protein. The mean protein concentration and phospholipase activity of the 2 kidneys of each rat was considered for statistical analysis which was carried out using the t-test for unpaired variables.

Results. The mean increase in body weight during the experimental period was 14±3 SEM g in DOCA treated and 25±5 SEM g in control rats, without a significant difference between the 2 groups (t = 1.5380). Blood pressure in DOCA treated rats was 115±1 SEM mmHg at the start and 136±3 SEM mmHg at the end of the study, and this increase was significant (t = 4.9766; $p < 0.001$). Blood pressure in control rats showed no significant variation (117±1 SEM mmHg at the start and the end of the study t = 0). Mean kidney protein concentration was 65±2 SEM mg in DOCA treated, and 66±3 SEM mg in control rats, without any difference between the 2 groups (t = 0.2514). Mean renal phospholipase activity was 1.916±0.113 SEM μeq/NaOH/min/mg protein DOCA treated, and 0.941±0.065 SEM μeq/NaOH/min/mg protein in control rats; the difference between the 2 groups was highly significant (t = 7.4433; $p < 0.001$).

Discussion. It has been repeatedly demonstrated that an excess of mineraloactive steroids increases kallikrein production[5,6,10], and that the generated kinins in turn promote prostaglandin release[4]. Recently Nasjletti et al[11] have confirmed that in DOCA treated rats the increase in prostaglandin production is kallikrein-dependent, since enzyme inhibition lowers prostaglandin release.

However, although the mechanism by which the kallikrein-kinin system influences prostaglandin synthesis has been postulated, it has never been demonstrated. From our data it appears that in DOCA treated rats there is a clear cut increase in renal phospholipase activity, and this enzyme is considered the key step in prostaglandin biosynthesis[7]. This increase in renal phospholipase activity might also affect the renin-angiotensin system, since the liberated lysophospholipids are know to inhibit the renin-angiotensinogen



Shows the kidney phospholipase activity of controls and DOCA treated rats.

reaction[12]. Finally, the increase in cellular lysolecithin might play an important role in the regulation of guanylate and adenylate cyclase activities[13].

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3 J.C. Mac Giff, N.A. Terragno, K.U. Malik and A.J. Lonigro, Circulation Res. *31*, 36 (1972).
4 J. Colina-Chourio, J.C. Mac Giff, M.P. Miller and A. Nasjletti, Br. J. Pharmac. *58*, 165 (1976).
5 H.S. Margolius, R.G. Geller, W. De Jong, J.J. Pisano and A. Syoerdsma, Circulation Res. *31*, 125 (1972).
6 R.G. Geller, H.S. Margolius, J.J. Pisano and H.C. Keiser, Circulation Res. *31*, 857 (1972).
7 H. Kunze and W. Vogt, Ann. N.Y. Acad. Sci. *180*, 123 (1971).
8 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
9 B. Baggio, S. Favaro, A. Antonello and A. Borsatti, Res. exp. Med. *169*, 77 (1976).
10 A. Adetuyibi and J.H. Mills, Lancet *2*, 203 (1972).
11 A. Nasjletti, J.C. Mac Giff and J. Colina-Chourio, Circulation Res. *43*, 799 (1978).
12 S. Sen, R.B. Smeby and F.M. Bumpus, Biochemistry *6*, 1572 (1967).
13 W.J. Shier, J.H. Baldwin, M. Nelson-Hamilton, R.T. Hamilton and N.M. Thanassi, Proc. nat. Acad. Sci. USA *73*, 1586 (1976).

Circadian variation of adrenocortical cyclic nucleotides (cyclic AMP and cyclic GMP) in hypophysectomized rats

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Summary. The existence of a circadian variation in the adrenocortical concentrations of cyclic AMP and cyclic GMP in male adult Wistar rats examined 10 days after hypophysectomy is demonstrated. The results suggest that the circadian variations of adrenocortical cyclic nucleotides observed previously in intact rats might not entirely depend upon pituitary corticotrophin.

A previous report[2] has examined the circadian rhythms in adrenocortical cyclic AMP(cAMP) and cyclic GMP (cGMP) as compared to the circadian variation of blood corticosterone. The question then arose whether the circadian variations of the adrenocortical cyclic nucleotides observed in intact rats were completely under the control of the pituitary. The present experiment is designed to answer that question.

Materials and methods. 52 hypophysectomized male Wistar rats weighing around 160 g each were supplied by IFFA-CREDO (Saint-Germain-sur-l'Arbresle, France). They had been hypophysectomized using the auricular technique under ether anaesthesia. The effectiveness of hypophysectomy was assessed by the following criteria: no weight gain, adrenal atrophy and undetectable blood corticosterone (below 0.2 µg/100 ml). The animals were used 10 days subsequent to operation. During this period they were maintained at a temperature of 22 °C with dry rat pellets and salt water available ad libitum. They were synchronized with natural day/light alternation. On the day of killing, sunrise occurred at 05.49 and sunset at 21.56 (local time = GMT + 2). The animals were sacrificed one by one at 10 min. intervals in series of 8-9 subjects. Each adrenal was decapsulated and its zona medullaris removed. The efficiency of this procedure in isolating only the fasciculata-reticularis zona has been assessed by histological examination[1]. The adrenocortical tissue was then lyophilized and weighed. The lyophilized tissues were extracted and the cyclic nucleotides determined by radioimmunoassay according to Cailla et al.[3,4].

The excellent specificity of the antibodies used (cross-reactivity between succinyl cAMP and succinyl cGMP was less than 0.01%) allowed direct measurement of the 2 cyclic nucleotides without their initial separation. Both conventional and Cosinor methods 5 were used for statistical analysis of the time series. Chronograms were drawn by plotting the studied variables (mean ± SEM for each group of animals) as a function of time (clock hours).

The differences between peaks and troughs have been checked using Student's t-test. The Cosinor method was used for both rhythm validation and quantification. The latter method involves computer programs in combination with the least-squares method in order to find the best fitting cosine function which approximates all data. With this method, a rhythm is validated when its amplitude (half of the peaktrough difference) differs from zero where $p < 0.05$. Furthermore, the Cosinor method takes the actual time of the death of each animal into account.

Results. Chronograms are shown in figures 1 and 2. It appears from the patterns of these chronograms that a circadian variation is present for adrenocortical cAMP and cGMP, since both curves are roughly a sine function.

Cosinor summary circadian rhythms in adrenocortical cAMP and cGMP in hypophysectomized rats

Variable tested	Rhythm detection	Mesor M ± SEM	Amplitude Mean	Range*	Acrophase h/min Mean	Range*
Cyclic AMP	$p < 0.005$	26.4 ± 2.8	2.5 [9.5]	1.6-3.5 [6.1-13.3]	05.24	03.57-06.54
Cyclic GMP	$p < 0.005$	0.46 ± 0.03	0.063 [13.7]	0.034-0.093 [7.4-20.2]	08.00	06.00-10.00

52 mature male Wistar rats hypophysectomized 10 days before. Synchronization with natural day-light. Sunrise at 05.49 and sunset at 21.56 (local time) on the day of killing. Sampling intervals $\Delta t = 4$ h. Trial period $\tau = 24$ h. The mesor or 24 h rhythm adjusted average is given in pmole/mg dry weight. The amplitude or half of the peak-trough difference is given in pmole/mg dry weight and as a percentage of the mesor in brackets. The acrophase or peak time is expressed in h and min. Phase reference is 00.00 (local midnight). * 95% confidence limits.