Effects of Pantothenate Deficiency on Steroid Hormone Secretion in Rats

I. Assessment of the State of Function of the Adrenal Cortex*

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Summary The effect of pantothenic acid (PA) deficiency on the adrenocortical function was investigated in young male rats fed a PA-deficient (PAD) diet over a period of about three months. Pair-weight (PW) and *ad libitum* (AL) fed rats served as controls. Basal 08.00 h (lights on) plasma levels of corticosterone were regularly found to be reduced in PAD and AL rats compared with those in PW animals. Investigation of the circadian rhythm of plasma corticosterone revealed that the elevated 08.00 h hormone concentrations of PW rats are due to a biphasic 24-h pattern of plasma corticosterone with a distinct glucocorticoid peak at lights on and another one at lights off (20.00 h), whereas PAD and AL rats only show one clear increase in plasma corticosterone levels at the beginning of the dark period. However, the evening glucocorticoid peak of the PAD rats was markedly higher than that of the PW and AL groups. Thus an adrenocortical overactivity is ascertained in PA deficiency which is presumably responsible for the adrenal hypertrophy generally observed in earlier studies on PA depletion in rats. Multiple examinations of the secretory capacity for corticosterone, each carried out half an hour after i.m. administration of 2.5 IU ACTH₁₋₂₄, revealed comparable plasma responses to supraphysiological ACTH stimulation in PAD, PW, and AL rats. Therefore we concluded that corticosterone secretion is not impaired in PA deficiency, at least not that elicited for short periods by adrenocortical overstimulation. Additional measurements of the adrenal cholesterol content and the circadian plasma levels of dehydroepiandrosterone-sulphate were carried out in all three diet groups. The respective results are discussed with regard to the stress situation produced by the experimental PA deficiency.

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Because of the widespread occurrence of pantothenic acid (PA) in foods, deficiency symptoms in humans have not been observed except in cases of severe malnutrition [1] and in volunteers fed either the antagonist Ω -methylpantothenic acid or a semisynthetic diet virtually free of pantothenate [2, 3]. Thus many of the known symptoms of a manifest PA deficiency have been seen only in experimental animals, which can be more rigorously controlled. In various species (rat, dog, pig) hypertrophy, congestion, and hemorrhagic alterations of the adrenal gland seem to occur regularly [4–6]. At an advanced deficiency stage also small areas of adrenal necrosis (predominantly in the inner cortex) up to subtotal or total cortical necrosis were seen in rats [4, 7, 8]. However, the stronger forms of adrenocortical necrosis were primarily observed in such rats fed PA-deficient diets with anti-vitamin supplements [8]. In the latter experiments, in which Ω -methylpantothenic acid was administered for at least several weeks, animals showed clear signs of severe adrenal cortical insufficiency with a drastically reduced capacity to secrete corticosterone.

A PA deficiency induced without administration of antivitamin rarely results in marked necrotic lesions of the adrenal cortex. But if young animals are used in such kinds of feeding experiments, a reduction in the coenzyme A content of many organs develops [9–11]. Since coenzyme A, the biologically active form of pantothenic acid, is involved in the synthesis of the steroid-hormone precursor cholesterol, it has been postulated that even in the prenecrotic stage of a manifest PA deficiency the corticosterone secretion is impaired [12–14]. However, the hormone measurements in these studies on PA deficiency induced without antivitamin administration, cannot be interpreted accurately, since no adequate adrenocorticotrophic hormone (ACTH) treatment was carried out in order to provoke a maximal secretory response of the prenecrotic adrenal gland *in vivo*. Furthermore, even the basal corticosteroid output is not yet well characterized in the literature with respect to the manifest prenecrosis stage. With this coenzyme A deficiency period especially the circadian rhythm of corticosterone deserves a precise investigation because the frequently occurring hyperemia and the hemorrhagic areas in the enlarged adrenal glands are clear signs of a severe stress situation [15]. And stress is known to modify the circadian secretory pattern of glucocorticoids. So this study was designed to clarify in more detail in which regard glucocorticoid output is altered by a manifest PA deficiency in the rat.

MATERIALS AND METHODS

Animals and housing conditions. In all studies described below very young rats (about 3 weeks of age) were used to ensure that tissue total CoA levels would

decrease during the course of several weeks when a strongly PA-deficient diet without antivitamin supplement was fed [9-11]. Male Wistar rats (specific pathogen free), weighing 35-40 g, were received from the Zentralinstitut für Versuchstierzucht, Hannover (FRG). The animals were randomly allocated to three groups, group pantothenic acid deficiency (PAD), group pair-weight (PW), and group *ad libitum* (AL) and were housed individually in cages with wire-mesh bottom to prevent stronger forms of coprophagy.

The cages were placed in a common animal room with controlled environmental conditions: temperature at $22 \pm 2^\circ\text{C}$, humidity within the range of 50-70%, and light on from 08.00-20.00 h. Body weight was recorded twice or during the initial period of study three times (groups PAD and PW) per week.

Diet and feeding schedule. The animals of group PAD received a semisynthetic PA-deficient powder diet similar as to that described by Hötzel [16], containing less than $50 \mu\text{g}$ pantothenate/kg food. This basic diet was supplemented with 50 mg calcium pantothenate per kg food to obtain the control diet for PW and AL rats. The basic PA deficient diet contained the following components (in percent): sucrose, 60; "vitamin free casein" (hot alcohol extracted) 20; rice starch, 8.5; vegetable oil (Mazola), 7; salt mixture [8], 4; choline, 0.2; *dl*-methionine, 0.2; and vitamin mixture [8], 0.1. All animals of the groups AL and PAD had unlimited access to water and the respective diet. Group PW rats had free access to water, but were diet restricted after 5 days of initial *ad libitum* feeding. The day after arrival of the animals was set as the first experimental day. From the sixth day food supply of PW rats was gradually reduced beneath the amount daily eaten by PAD rats, in order to adapt the growth of group PW to that of group PAD. For this, during the first few weeks food consumption of PAD rats was measured daily and later on twice weekly. The consumption rate (for each 24-h period) was determined by subtracting, from a preweighed amount given at 11.00 h, the quantity remaining in the food bowls 24 h later. The average consumption/day was calculated after correction had been made for the lost food collected beneath the cages. Food intake of AL rats was less frequently recorded in the same way. The control diets for AL and PW rats were also supplied between 11.00 and 12.00 h colony time each day.

Study I. Deficiency symptoms and peripheral plasma levels of corticosterone. Forty-two rats (14 PAD, 14 PW, 14 AL) were maintained for about 3 months on the PA-deficient or the PA-supplemented diet as described above. During this period all rats were subjected to 3 blood samplings scheduled at 1-month intervals. In 7 animals of each dietary group blood was taken on day 21, 50, and 78; in the remaining rats blood samples were collected on day 35, 65, and 95. Besides regular measurements of circulating hormone levels, on day 65 also the total cholesterol in plasma of AL, PW, and PAD rats was determined. In addition, on the 28th experimental day urinary excretion of PA and creatinine was investigated in those rats, which were subjected to a first blood collection one week before. For this purpose the animals were kept individually over a 24-h period in screen bottom

metabolism cages which were tightly fitted on urine collection funnels. Thus, it was possible to collect the urine without marked faeces contamination. At the end of the study—immediately after the last blood withdrawal on day 95—rats were killed and both adrenals of each animal were quickly removed, carefully freed of connective tissue, weighed, and stored frozen until assay of cholesterol content was carried out.

Study II. Adrenal corticosterone responses to exogenous ACTH stimulation.

The capacity for corticosterone secretion following a short-term stimulation test with ACTH was investigated after 21, 35, 50, and 85 days on the respective diet in a group of 7 AL, 7 PW, and 7 PAD rats. At each of these experimental days at 08.00 h ("lights on") all 21 animals were injected i.m. with 2.5 IU of synthetic ACTH₁₋₂₄ (Synacthen, Ciba Geigy). Blood was taken half an hour later.

Study III. Circadian rhythms of corticosterone and dehydroepiandrosterone-sulphate. In order to determine the effects of a manifest PA deficiency on the circadian rhythms of corticosteroids, 162 male Wistar rats (about three weeks of age) were placed on the PA-deficient and PA-supplemented diets (AL, PW) for nearly 7 weeks. The animals ($n=54$) of each dietary regime were subdivided into 9 groups for taking blood samples only once per animal. Throughout a 24-h period starting off with the 48th experimental day, groups of AL, PW, and PAD rats were subjected to a blood withdrawal at 12.00, 15.00, 18.00, 21.00, 24.00, 03.00, 06.00 or 09.00 h with one overlapping period at 12.00 h. The overlapping period was included in order to check for possible appearance of serial effects. The latter may arise from repeated disturbance of the colony, caused by taking out animals every 3 h from the common animal room. A red light was used for cage identification during the dark period [17]. Blood was taken in an adjacent room.

Blood collecting. Blood was taken by orbital sinus puncture under light ether anesthesia. This procedure took place within 2.5 min after removal of each rat from the cage. Blood was collected into ice-cold EDTA-containing tubes. EDTA-plasma was stored at -22°C . In rats subjected to subsequent blood collections only about 2 ml blood was removed.

Analytical methods. Corticosterone was assayed by a fluorimetric method which is based on the fluorescence of this steroid in sulfuric acid. In brief, the following extraction procedure was performed prior to hormone measurement: 50 μl redistilled water was added to 200 μl of each plasma sample. The diluted samples were extracted with 5 ml methylene chloride (vortex-mixing for 2 min). Phases were separated by centrifugation at $1500\times g$ for 5 min. After removing the upper aqueous layer an aliquot of 4 ml of the organic phase was withdrawn and shaken for 30 s with 1.2 ml of fluorescence reagent (7 volumes of concentrated sulfuric acid, added to 3 volumes of ethanol for fluorescence-spectroscopy, Merck, No. 973). Corticosterone standards and a water blank were run through the same procedure. Preparation of standards and measurement of fluorescence were performed according to Van der Vies *et al.* [18]. The detection limit for corticosterone standard solutions was found to be 2 $\mu\text{g}/100\text{ ml}$. The contribution of other

substances in plasma to the total fluorescence was determined in samples from dexamethasone treated rats. A mean non-specific plasma fluorescence of $2.8 \mu\text{g}/100 \text{ ml}$ was detected (range: 2.3 to $4.0 \mu\text{g}/100 \text{ ml}$). Standard addition ($10 \mu\text{g}$ corticosterone/ 100 ml rat plasma) prior to extraction showed quantitative (89–104%) recovery. The within-assay variability was 8% and the between assay variability was 9% at $50 \mu\text{g}/100 \text{ ml}$. A survey of the relative fluorescence intensities of related steroids compared to corticosterone or cortisol is given by Mattingly [19]. A method comparison of the assay described here (X) and a fluorimetric determination of CH_2Cl_2 -extracted corticosterone after additional separation of steroids by celite column chromatography (Y)—carried out with 8 rat plasma samples—yielded the following correlation: $Y = 1.05X - 3.77 \mu\text{g}/100 \text{ ml}$ ($r = 0.984$). Celite microcolumns were obtained from Radioassay Systems Laboratories, Carson, California. Plasma for reliability experiments was derived from quiescent and stressed male rats.

Plasma levels of dehydroepiandrosterone-sulphate (DHEA-S) were determined by radioimmunoassay as described previously [20]. The quantification of PA in rat urine was carried out with a gas chromatography (GC) method following purification by ion-exchange chromatography, derivatisation of PA to pantolactone and CH_2Cl_2 -extraction of the lactone prior to GC-measurement [21]. Creatinine in urine (a), and total cholesterol in plasma (b) and adrenals (c) were measured by colorimetric methods with enzyme kits of (a) Merck, FRG (No. 3385); (b) Boehringer Mannheim, FRG (No. 290319); (c) Boehringer Mannheim FRG (No. 139050, "cholesterol in foodstuffs"). As for the determination of cholesterol in adrenals both glands of each animal were minced with a blade. The esterified sterol-fraction was hydrolysed by methanolic potassium hydroxide solution (0.5 mol/liter) heated under a reflux condenser, and total cholesterol was extracted twice with isopropanol as specified in the kit instructions for the measurement of cholesterol in foodstuffs.

Statistics. Data statistically analysed without logarithmic transformation are presented as means $\pm$ standard error, unless otherwise stated. A transformation of raw data to logarithms was performed to stabilize variance or to obtain normal distributions of non-normal distributed variables. In these cases geometric means as well as the respective 95%-confidence limits are given and/or means $\pm$ SE in the logarithmic scale. When parameters were investigated in only two diet groups, student's t -test for unpaired data was used to test differences in the mean values. Otherwise one-way or two-way analysis of variance (ANOVA) was performed for multiple comparisons, followed by the Tukey or Scheffé test if the ANOVA was significant [22].

RESULTS

Study I. Deficiency symptoms and peripheral plasma levels of corticosterone

Animals fed the deficient diet consumed markedly less food throughout the

Fig. 1. Growth curves for AL, PW, and PAD rats throughout the feeding experiment of study I. Mean $\pm$ SD; $n=14$ rats per diet group. ■, AL; $\triangle$, PW; ●, PAD.

experimental period than did the *ad libitum* fed controls. The mean food consumption of AL rats was 16.7 g/day, whereas PAD and PW rats ingested only 12.2 and 9.2 g food/day, respectively. So the caloric intake of PW control rats had to be restricted to about 75% of the amount eaten by group PAD animals in order to achieve comparable growth rates in both groups. As depicted in Fig. 1, no clear-cut differences in body weight occurred between the PAD animals and the PW controls during the study. At the beginning of the second experimental week a significant slower gain in body weight was distinguishable for the PAD rats compared with those fed the PA-containing diet *ad libitum*. Although the effect of PA deficiency on body weights was very pronounced in the course of the feeding experiment, PAD rats showed a steadily increasing growth curve (Fig. 1). In the following studies food consumption and growth curves of the experimental animals were nearly the same as depicted here. Therefore these gross metabolic data are presented only once (for the animals of this study).

Besides weight depression, more characteristic signs of PA deficiency became evident after the animals had been on the diet for 25–30 days. These consisted of porphyrin staining of the hair, dermatitis, and loss of fur from the head and neck. Nearly one third of the PAD rats showed at least one of these symptoms to a marked degree. After more than 8 weeks on the deficient diet these macroscopic symptoms gradually disappeared in the greater part of the PAD group rats. This phenomenon was accompanied by an increased frequency of coprophagy in

Table 1. Urinary excretion of pantothenic acid and creatinine.

	AL (n=7)	PW (n=7)	PAD (n=7)
Pantothenic acid ^a	5.78	3.62	0.58
($\mu\text{g}/\text{mg}$ creatinine)	3.46–9.64 (0.762 ± 0.091)	2.05–6.39 (0.559 ± 0.101)	0.38–0.89 (-0.235 ± 0.075)
Creatinine ^b (mg/d)	6.15 ± 0.25	3.90 ± 0.17	3.86 ± 0.37

Because of the comparability with data of earlier investigations [8], PA excretion is related to mg urinary creatinine. Results are listed as geometric means with their respective confidence limits. In addition means $\pm$ SE are given in the logarithmic scale in parentheses.

^a(F(2/18)=34.6), PAD vs. PW, $p < 0.001$; PAD vs. AL, $p < 0.001$; ^b(F(2/18)=22.4), AL vs. PW, $p < 0.001$; AL vs. PAD, $p < 0.001$.

Table 2. Total cholesterol in plasma and adrenals, and relative adrenal weights.

	AL (n=7)	PW (n=7)	PAD (n=7)
Plasma cholesterol ^a	121.2 ± 6.3	71.9 ± 3.1	74.5 ± 8.3
(mg/100 ml)			
Adrenal cholesterol ^b	$1,305.0 \pm 108$	$3,581.0 \pm 124$	$1,366.0 \pm 114$
(mg/100 g WW) ¹			
Adrenal weight ^c	16.5 ± 1.03	16.6 ± 0.90	19.5 ± 0.77
(mg/100 g BW)			

¹(mg cholesterol/100 g wet weight). ^a(F(2/18)=19.7), AL vs. PW, $p < 0.001$; AL vs. PAD, $p < 0.001$. ^b(F(2/18)=125.2), PW vs. AL, $p < 0.001$; PW vs. PAD, $p < 0.001$. ^c(F(2/18)=3.6, $p < 0.05$), PAD vs. PW, $p < 0.1$; PAD vs. AL, $p < 0.1$.

deficient animals. Measurement of urinary PA excretion after 4 weeks on the respective diet confirmed that a severe depletion of this vitamin had developed before the first macroscopic deficiency signs occurred (Table 1).

Daily PA excretion and consequently the excretion of this vitamin related to mg urinary creatinine were drastically reduced in PAD compared with AL and PW rats (Table 1). On the other hand urinary creatinine values were indistinguishable between animals of group PAD and PW, thus reflecting an identical muscular mass in both kinds of restrictedly fed rats. Cholesterol blood levels measured on the 65th experimental day are presented in Table 2. As already stated for urinary creatinine excretion the circulating plasma cholesterol was also nearly identical in PW and PAD rats, and as a sign of chronic undernutrition it was found to be markedly reduced in comparison with that in AL animals. In contrast to this adrenal cholesterol content assayed at the end of the study varied in a way which cannot be attributed to the fact that the nutritional status with regard to growth rates is impaired in PW and PAD rats to the same degree. Total cholesterol in adrenals of PW rats was clearly elevated above the levels of PAD and AL animals (Table 2). However, the relative adrenal weights (expressed as mg of adrenal tissue per 100 g body weight) of PAD rats revealed a tendency toward hypertrophy (one-way analysis of variance: ($p < 0.05$; Table 2).

Basal corticosterone plasma levels of PAD, PW, and AL animals are given in Fig. 2. The mean corticosterone concentrations in the circulation of the PAD

Fig. 2. Basal 08.00 h plasma levels of corticosterone (in the logarithmic scale) after different periods under the AL, PW, and PAD feeding regime. Each bar represents the geometric mean ($\pm$ SE) of $n=7$ animals; day 21: ($F(2/18)=5.58$); day 35: ($F(2/18)=7.71$); day 50: ($F(2/18)=14.10$); day 65: ($F(2/18)=24.98$); day 78: ($F(2/18)=17.30$); day 95: ($F(2/18)=5.24$); * $p<0.05$, ** $p<0.01$, *** $p<0.001$. AL; PW; PAD.

group were almost the same as those of the AL controls and significantly less than those of PW rats at all dates of examination. As can be seen from Fig. 2, the glucocorticoid levels of the PW group at lights on increased steadily during the first 7 weeks, reached peaks of nearly identical magnitude on days 50, 65, and 78, and decreased at the end of the study, thus indicating a stress situation with a varying degree of adrenal activity during the feeding experiment. Since these hormone measurements only reflect the secretory activity of the adrenal cortex during a short circadian phase in the morning, no conclusions can be drawn with regard to the overall stress situation of rats under a respective feeding regime.

Study II. Adrenal corticosterone responses to exogenous ACTH stimulation

The responses of the circulating levels of corticosterone to exogenous ACTH (short term stimulation test) in PAD, PW, and AL rats after 3, 5, 7, and 12 weeks on the respective diet are depicted in Fig. 3. At each of the 4 short-term stimulation tests and in all groups, mean plasma corticosterone concentrations of about $70 \mu\text{g}/100 \text{ ml}$ were achieved, thus indicating a clear incremental response of adrenal glucocorticoid secretion when compared with the basal steroid levels illustrated in Fig. 2. No impairment in the secretory capacity for corticosterone was detectable in PA deficiency after this kind of short term exposure to a supraphysiological

Fig. 3. Responses of plasma corticosterone to short-term ACTH stimulation (blood collection: half an hour after i.m. administration of 2.5 IU ACTH₁₋₂₄; $n=7$ rats per diet group). $\square$, AL; $\square$ (stippled), PW; $\square$ (checkered), PAD.

Table 3. Results of the two-way analysis of variance on the circadian rhythm of corticosterone.

Source of variation	Sums of squares	DF	Variance	F	p
Total	15.896	143	0.111		
Diet	1.731	2	0.865	15.575	$p<0.001$
Time	3.587	7	0.512	9.224	$p<0.001$
Diet $\times$ Time	3.911	14	0.279	5.028	$p<0.001$
Error ("R")	6.667	120	0.056		

Table 4. Mean corticosterone plasma levels during a 24-h period.

	AL ($n=48$)	PW ($n=48$)	PAD ($n=48$)
$\bar{x}_g$ ($\mu\text{g}/100\text{ ml}$)	12.0	22.3	16.0
95%-confidence limits	9.7-14.9	19.2-25.9	12.4-20.7
$\bar{x}_{(10g)} \pm \text{SEM}$	1.080 ± 0.0462	1.348 ± 0.0326	1.204 ± 0.0555

Tukey test: PAD vs. AL, $p<0.05$; PW vs. AL, $p<0.001$; PAD vs. PW, $p<0.01$.

ACTH dose. The slightly higher corticosteroid responses of PAD rats seen on days 21 and 35 were found to be statistically insignificant.

Study III. Circadian rhythms of corticosterone and dehydroepiandrosterone sulphate

Analysis of variance revealed significant differences in the 24-h mean levels of corticosterone with regard to the different feeding regimes (Table 3, factor; diet, $p<0.001$). Respective mean serum levels of corticosterone for AL, PW, and PAD rats are given in Table 4. The multiple comparison procedure of Tukey yielded significantly higher corticosterone 24-h means in PW ($p<0.001$) and PAD ($p<$

Fig. 4. Twenty-four hour patterns of plasma corticosterone levels in AL, PW, and PAD rats determined at the end of the 7th experimental week. Hormone concentrations are depicted as arithmetic means $\pm$ SE as it is usually done for the graphic presentation of the circadian rhythm of corticosterone. $\times$, AL; $\bullet$, PW; $\triangle$, PAD.

0.05) rats than in the AL group, thus confirming the existence of a stress situation for the underweight animals. In addition PW rats showed an increased mean corticosterone blood level compared with PAD rats.

As can be seen from Fig. 4, the circadian corticosterone pattern of both "stress groups" (PW and PAD) didn't run parallel. This comes out statistically as significant ($p < 0.001$) interactions between the factors diet and time (Table 3). The circadian pattern of plasma corticosterone of PW rats revealed a biphasic rhythm with one peak in the morning and another one at the beginning of the dark period (Fig. 4). PAD and AL rats, however, only showed one clear corticosterone maximum at 21.00 h.

Significant 24-h periodicity in plasma levels of corticosterone could be demonstrated statistically (test of simple main effects) in AL ($F(7/120) = 8.41$; $p < 0.001$), PW ($F(7/120) = 3.30$; $p < 0.02$), and PAD rats ($F(7/120) = 7.43$; $p < 0.001$). Contrary to the corticosterone measurements in AL and PAD group animals, the morning glucocorticoid levels of PW rats at 06.00 and 09.00 h did not differ significantly from their 21.00 h peak. This again stresses the already mentioned bimodal rhythm in the 24-h pattern of corticosterone as a feature of the PW rats. Particularly at 21.00 h the variations of the corticosterone measurements of all three diet groups were similar in magnitude, and in addition just at this time a very low variance of the experimental error (variance "R" = 0.014) was found in comparison with the variance "R" of all circadian measurements (Table 3, variance "R" = 0.056). So a separate one-way analysis of variance was carried out for the

21.00 h blood levels in order to eliminate the stronger residual variation caused by the corticosterone measurements before and after the evening peak. This procedure revealed a significantly ($p < 0.025$; Tukey test) elevated 21.00 h glucocorticoid serum level in PAD compared with PW and AL rats ($F(2/15) = 8.6$). Thus in PA deficiency the physiologically occurring evening corticosterone peak shows an increase in amplitude. In contrast to corticosterone, neither in AL and PW rats nor in PAD animals a clear cut circadian periodicity could be detected for the adrenal androgen DHEA-S. Basal blood levels of this steroid sulphate were found in the range of approximately 0.3–0.4 ng/ml in all diet groups and at each time of the day. These low androgen blood levels correspond well to earlier measurements of DHEA-S in unstressed rats [20, 23]. Because of the almost identical androgen sulphate concentrations (irrespective of the time of day) in the circulation of AL, PW, and PAD rats, the 24-h patterns of plasma DHEA-S have not been presented.

DISCUSSION

The higher food intake of PAD rats compared with that of identically growing PW control animals can be interpreted according to Reibel *et al.* [24] as the result of an impaired intestinal transport of ions and substrates in PA deficiency. Although the coenzyme A content of liver, adrenal, and other metabolically important organs is reduced in young rats which soon after weaning are fed a PA-deficient diet for at least 3 weeks [9–11], no decrease in total plasma cholesterol could be detected in corresponding PAD rats (study I). This indicates that the moderate coenzyme A reduction induced without antivitamin administration still permits a sufficient hepatic cholesterol synthesis to cover the decreased need for circulating cholesterol in underweight animals. In the rat as in several other mammalian species the adrenal steroid hormones are mainly derived from plasma cholesterol [25, 26]. This is compatible with almost identical corticosterone responses to exogenous ACTH as assayed for all diet groups in study II.

Regarding the short-term stimulation with ACTH we can conclude that in a manifest PA deficiency induced without antivitamin administration the cholesterol supply of the adrenal gland is sufficient so as to allow a maximum serum-corticosterone response not differing from that in control animals.

The investigation of the circadian rhythm of plasma corticosterone (study III) shows that the regularly elevated 08.00 h corticosterone blood level in PW rats (study I) reflects a distinct glucocorticoid rise with a peak between 06.00 and 09.00 h. This early increase in adrenal secretory activity can be interpreted as the result of the restricted food supply during the light period [27–29] and according to Honma *et al.* [30] it is called “prefeeding corticosterone peak.” PW rats received their restricted feed each day at 11.00 h in the morning. It is generally accepted that the daily rhythm of plasma corticosteroid concentration can be entrained by several environmental factors, including restricted feeding cycles [29, 31]. Presumably a similar “prefeeding corticosterone peak” as in PW rats is responsible for the

lower corticosterone secretion ascertained by Goodman [13] for PA-deficient compared with restrictedly fed control rats in a study without ACTH administration. Owing to the biphasic pattern of plasma corticosterone (second rise in the evening) seen in PW animals, the adrenocortical stress activity of this control group varies from the stress situation discernible in PA-deficient animals. PAD rats reveal only one clear corticosterone peak at 21.00 h which, however, is significantly elevated in comparison with the corresponding measurement results obtained in PW and AL rats. From these data we presume that the adrenal hypertrophy regularly observed in PA deficiency [7, 8, 12] is produced by an higher ACTH stimulus responsible for the clearly elevated amplitude of the physiologically occurring evening corticosterone peak. In addition this phenomenon again shows that corticosterone secretion is not impaired in PA deficiency, at least regarding for short periods of adrenocortical overstimulation.

The investigation of the 24-h pattern of plasma DHEA-S revealed that no clear circadian periodicity exists for this hormone in the circulation of rats. A corresponding observation has been made with regard to the very high DHEA-S plasma levels in human beings [32, 33]. This phenomenon was explained by the long half-life of DHEA-S in man. However, the same can not hold true for the rat, since in this species the metabolic clearance rate of DHEA-S has been found to be similarly high as that of unconjugated steroids [34]. As is evident from the experimental data, the daily increases in endogenous ACTH secretion responsible for the respective corticosterone peaks are not strong enough to stimulate adrenal DHEA-S output markedly. Even in PAD rats which exhibited an elevated amplitude in the evening corticosterone rise (as a sign of a temporarily stronger ACTH stimulus) no effects on DHEA-S plasma levels were discernible. Consequently the question is raised as to whether the absence of an increase in PAD rat's evening DHEA-S plasma levels could be the result of an impaired secretory capacity for adrenal androgens. This distinct aspect of adrenal steroid metabolism will be dealt with in a separate investigation on PA deficiency.

In accordance with the fact that continuous stress results in an increased cholesterol content of the adrenal gland [35, 36], occurring obviously in order to prepare this endocrine organ for additional secretory stimuli, PW rats showed a markedly elevated adrenal cholesterol deposition compared with AL animals. However, no such adaptation of adrenal steroid metabolism to stress was detectable in the PAD group which also exhibited a statistically confirmed adrenocortical overactivity. Whether the lower adrenal cholesterol content is definitely a specific symptom of PA deficiency as proposed in earlier publications [37, 38] has to be investigated in more detail in future studies, for example after a synchronization of the different stress situations of PAD and PW control rats. Because distinct consequences of divergent forms of stress cannot be completely excluded with regard to adrenocortical cholesterol metabolism, no final conclusions should be drawn here.

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