Brit. Pharmac. (1971), **42**, 595–602.

Suppression of adrenocorticotrophic activity in the ascorbic acid deficient guinea-pig

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Summary

1. Adrenocortical hyperactivity caused by a marked increase in circulating corticotrophin occurred in guinea-pigs on a diet deficient in ascorbic acid.

2. Betamethasone prevented the rise in the blood ACTH concentration in scorbutic animals and also the increased steroid production per gramme adrenal tissue *in vitro*. It diminished the adrenal hypertrophy and partially suppressed the rise in plasma cortisol.

3. Ninety minutes after the injection of ascorbic acid corticotrophin could no longer be detected in the plasma of scorbutic animals.

4. Neither the survival time nor the weight loss was affected by betamethasone treatment.

Introduction

It is now considered unlikely that ascorbic acid is essential for the synthesis and release of adrenal cortical steroids. There is, however, some evidence that the vitamin may have a functional importance in corticosteroid metabolism as many of the symptoms of scurvy are similar to those of adrenocortical insufficiency (Giroud, Martinet & Bellon, 1941 ; Lockwood & Hartman, 1933). Corticosteroids have also been claimed to be beneficial in the treatment of scurvy (Hyman, Ragan & Turner, 1950; Herrick, Mead, Egerton & Hughes, 1952). An enormous increase in pituitary-adrenocortical activity occurs in the guinea-pig in the terminal stages of scurvy and appears to be due mainly to the non-specific stress imposed by the vitamin deficiency (Hodges & Hotston, 1970). This work has been extended, and described here are the results of experiments in which the effect of the semi-synthetic corticoid, betamethasone, on the hypercorticism which develops in the scorbutic guinea-pig was studied.

Methods

Animals

One hundred and sixty female guinea-pigs weighing 250–350 g were used. They were obtained from the same source and maintained on a normal or an ascorbic acid deficient diet as described previously (Hodges & Hotston, 1970).

Drugs

Betamethasone disodium phosphate (Betnesol, Glaxo) was dissolved in tap water in concentrations of 2 μg/ml and 20 μg/ml and given in place of drinking water. Fresh solutions were prepared daily and the volumes ingested were recorded.

Ascorbic acid (Redoxon, Roche Products Ltd) solution containing 200 mg/ml was injected intraperitoneally in volumes of 5 ml/guinea-pig.

Blood samples

Blood was collected from the trunks of decapitated animals into chilled tubes containing approximately 10 IU heparin, centrifuged immediately and the plasma was stored overnight at −12°C.

Adrenal glands

Right adrenal glands, removed immediately after the collection of the blood samples, were prepared for the determination of their cortisol content (Hodges & Hotston, 1970) and left adrenals for measuring the cortisol production *in vitro* using a modification of the method of Bakker & de Wied (1961) as described by Flack (1970).

Plasma corticotrophin (ACTH) and cortisol concentrations

These were estimated as described by Hodges & Vernikos (1959) and Zenker & Bernstein (1958), respectively.

Results

Table 1 shows the fluid and steroid intake and Fig. 1 the growth rates of guinea-pigs on normal diets and diets deficient in vitamin C, with and without betamethasone in the drinking water. The ingestion of large quantities of the steroid had no significant effect on growth rate. Neither the loss of weight in the animals deficient in ascorbic acid nor their survival time (18–25 days) was affected by betamethasone. The adrenal hypertrophy (Table 2) resulting from ascorbic acid deficiency was unaffected by the smaller but diminished by the larger dose of betamethasone. Adrenal weights were expressed as a proportion of the greatest body weight attained during the course of the experiment instead of the final body weight because of the marked difference between the growth rates of the normal guinea-pigs and guinea-pig deficient in ascorbic acid.

The plasma cortisol concentrations 21 days after the commencement of the experiment are shown in Fig. 2. Vitamin C deficiency caused a marked elevation in plasma cortisol which was reduced but not completely suppressed by betamethasone. The concomitant rise in adrenal cortisol concentration was also prevented by the

TABLE 1. *Fluid and betamethasone intake of normal guinea-pigs and guinea-pigs deficient in ascorbic acid*

	Normal					Ascorbic acid deficient				
		Betamethasone					Betamethasone			
	Tap water	2 μg/ml		20 μg/ml		Tap water	2 μg/ml		20 μg/ml	
	Total vol	Total		Total		Total vol	Total		Total	
Days	(ml)	Vol (ml)	Steroid (mg)	Vol (ml)	Steroid (mg)	(ml)	Vol (ml)	Steroid (mg)	Vol (ml)	Steroid (mg)
7	614	811	1·6	850	17·0	730	1,012	2·0	983	19·7
14	1,197	1,550	3·1	1,912	38·3	1,313	1,794	3·6	2,323	46·5
21	1,904	2,350	4·7	2,888	57·8	1,522	2,084	4·2	2,913	58·3

All figures are expressed as (mean value/animal)/7, 14 or 21 days; ten–fourteen animals in each group.

higher concentration of the steroid (Table 3). The plasma cortisol concentration in animals on the normal diet was not affected by betamethasone, but the adrenal concentration was reduced.

Cortisol production per hour was studied *in vitro* for 3 h using adrenal glands obtained 21 days after the commencement of the experiment, and the results are shown in Fig. 3. In animals receiving adequate amounts of ascorbic acid, a basal rate of cortisol production occurred in the second and third hour which was always less than in the first hour of incubation. This initial cortisol production rate in normal guinea-pigs was reduced to the basal rate by the larger dose of betamethasone.

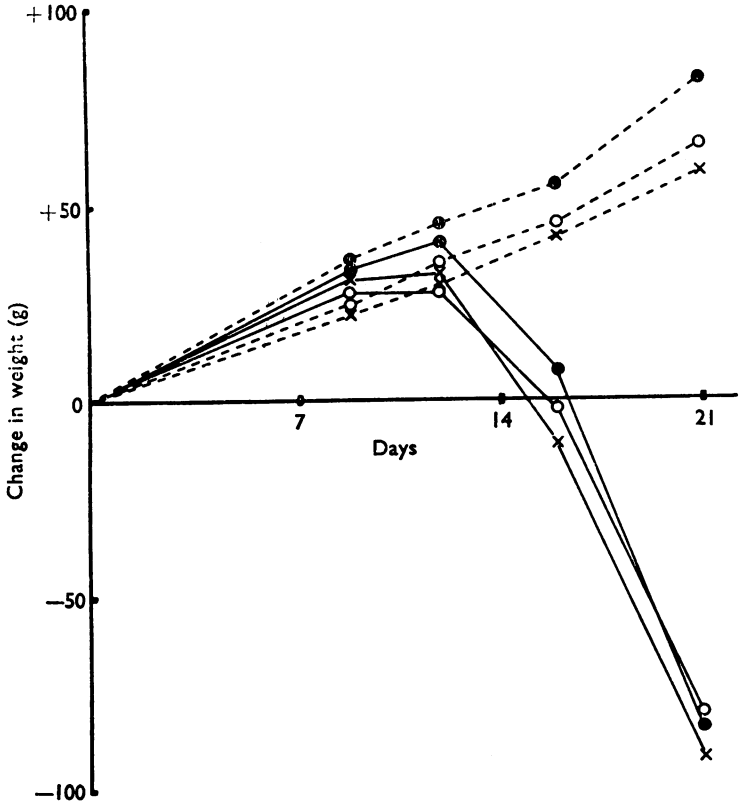

FIG. 1. Effect of betamethasone on changes in body weight in guinea-pigs on a normal diet (- - - - -) and a diet deficient in ascorbic acid (——). Controls (X); betamethasone, 2 μg/ml (●); betamethasone, 20 μg/ml (○). Eighteen animals per group.

TABLE 2. *Effect of betamethasone on adrenal weight in normal guinea-pigs and guinea-pigs deficient in ascorbic acid**

Beta-methasone (μg/ml) in drinking water for 21 days	Mean adrenal weight at end of experiment (mg±S.E.)		Mean body weight (g) at end of experiment		Ratio adrenal weight (mg)/greatest body weight† (100 g)	
	Normal	Scorbutic	Normal	Scorbutic	Normal	Scorbutic
0	124·5±9·1	301·0±11·3	380	291	33·0	72·8
2·0	170·1±9·6	298·6±18·1	456	298	37·3	71·9
20·0	107·9±2·2	170·6±12·3	392	334	27·5	38·7

*Six animals in each group. †Greatest body weight attained during the course of the experiment.

Cortisol production *in vitro* was significantly elevated in adrenal glands from guinea-pigs on the diet deficient in ascorbic acid. This effect of ascorbic acid deficiency was suppressed by betamethasone treatment and the amounts of cortisol produced *in vitro* by the adrenals of normal and scorbutic animals receiving the larger dose of the steroid were not significantly different.

Table 4 shows the plasma corticotrophin concentrations in the experimental animals. The results are expressed both as adrenal ascorbic acid depletions in assay rats (Hodges & Vernikos, 1959) and as ACTH/100 ml plasma. ACTH was just detectable in the blood of normal guinea-pigs. There was no rise in plasma ACTH after 14 days but an approximately 30-fold increase after 21 days of ascorbic acid

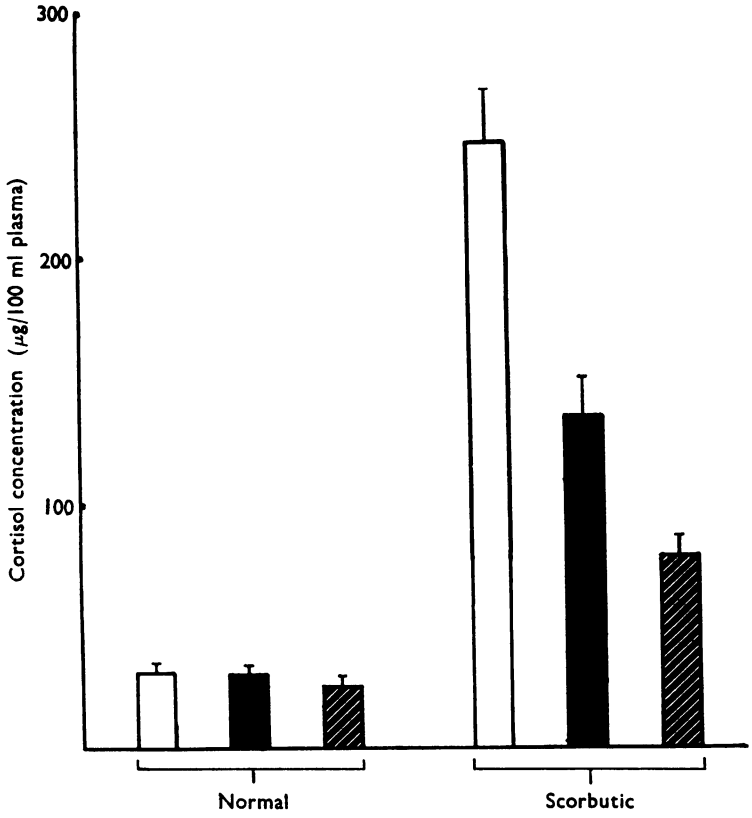

FIG. 2. Effect of betamethasone on plasma cortisol concentrations in normal and scorbutic guinea-pigs. Controls (□); betamethasone, 2 μg/ml (■); betamethasone, 20 μg/ml (▨) for 21 days. Each result is the mean of ten–twelve determinations and the vertical bars indicate the S.E.

TABLE 3. *Effect of betamethasone on adrenal cortisol concentrations in normal guinea-pigs and guinea-pigs deficient in ascorbic acid*

Betamethasone (μg/ml) in drinking water for 21 days	Cortisol concentration (mg/100 g adrenal tissue±standard error)	
	Normal	Scorbutic
0	2·2±0·2	3·6±0·4
2·0	2·3±0·2	3·4±0·3
20·0	1·7±0·1	2·2±0·2

Six animals in each group.

deficiency. This marked elevation in circulating corticotrophin was completely prevented by the betamethasone treatment, or by the injection of ascorbic acid 90 min before collection of the blood samples.

Discussion

Betamethasone was ineffective both in prolonging survival time and in preventing weight loss in guinea-pigs deficient in ascorbic acid. Our observations are in agreement with those of Clayton (1954) who found that the naturally occurring adrenal cortical hormones do not produce beneficial effects in scorbutic animals, and are in contrast with those of Lockwood, Swan & Hartman (1936) and Ratsimamanga (1944) who reported that they do.

The elevated circulating cortocotrophin and increased cortisol production *in vitro* indicated that the adrenocortical hyperactivity in the scorbutic guinea-pig is due to increased pituitary adrenocorticotrophic activity and confirmed the work of Clayton,

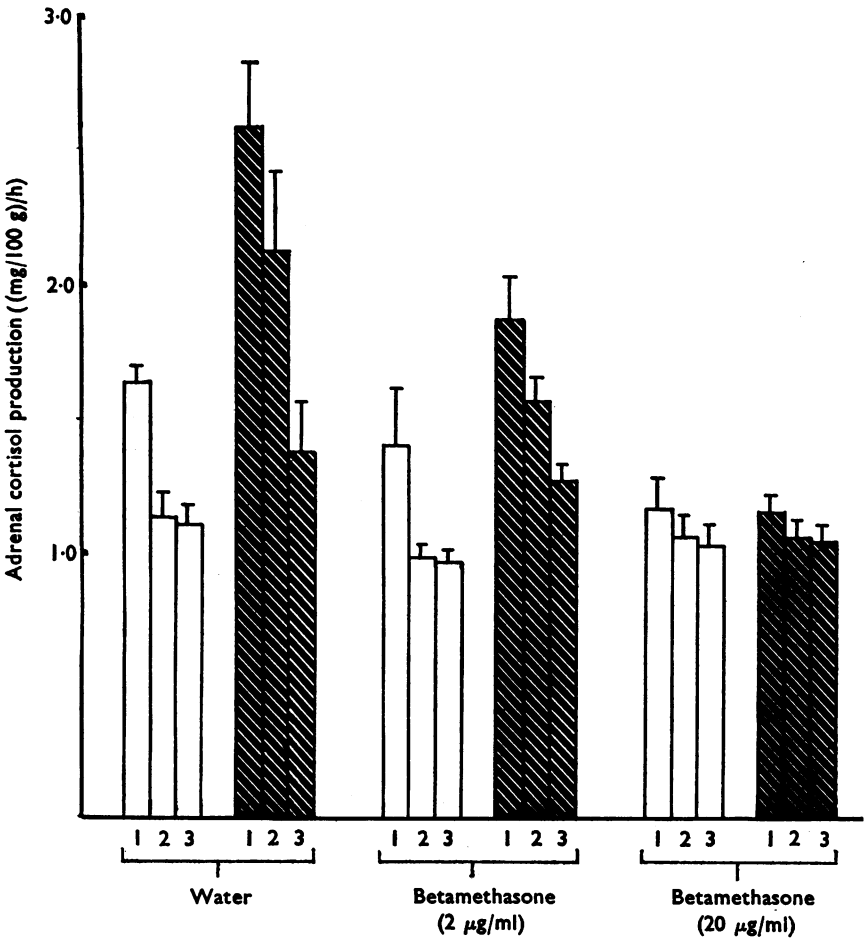

FIG. 3. Effect of betamethasone on cortisol production per hour by adrenal glands *in vitro* from normal (□) and scorbutic (▨) guinea-pigs. Each result is the mean of six determinations and the vertical bars indicate, the S.E. The columns in each group represent first, second and third hour of incubation.

Hammant & Armitage (1957). However, the occurrence of such remarkably high concentrations of the trophic hormone in the blood of the scorbutic animals, and the presence of easily detectable concentrations in normal animals cast doubt on the specificity of our assay technique, particularly as heterologous blood causes delayed adrenal ascorbic acid depletion in rats in which the mobilization of endogenous ACTH has been inhibited by corticoid treatment (Hodges & Jones, 1962). However, the validity of the assay was verified using plasma incubated for 24 h at 37°C which destroys its adrenal ascorbic acid depleting activity.

The development of high concentrations of circulating corticotrophin in guinea-pigs deficient in ascorbic acid was prevented by betamethasone. Other signs of increased hypothalamo-pituitary-adrenocortical activity, such as adrenal hypertrophy and increased corticoidogenesis *in vitro*, were also reduced but the blood cortisol concentration was still elevated. Most of the evidence in the literature (Banerjee & Singh, 1957; Clayton & Prunty, 1951; Hodges & Hotston, 1970) suggests that this could not have been the result of impaired steroid metabolism in ascorbic acid deficiency. It may have been a reflection of the greater ratio of the adrenal weights to body weights. A reduction in the blood volume (Harman & Kordisch, 1945) may also contribute to the increase in the plasma cortisol concentrations. The effect of betamethasone was not unexpected since it is well known as a potent inhibitor of ACTH release (Hodges & Mitchley, 1970). On the other hand, the mechanism by which ascorbic acid dramatically reduces the high concentration of ACTH in the blood of guinea-pigs deficient in ascorbic acid, as was also shown by Prunty, Clayton & Hammant (1957), is less clear. However, in certain circumstances, ascorbic acid can inhibit ACTH release as do corticoids (Dugal & Thérien, 1949), and ascorbic acid and corticoids together are more effective than corticoids alone in reducing pituitary adrenocorticotrophic overactivity in adrenalectomized rats (Hodges & Vernikos, 1962). Other evidence (Bacchus, 1954; Bacchus, Heiffer & Altszuler, 1952; Booker, Dacosta, Tureman, Froix & Jones, 1955; de Wied, 1953) also suggests that the vitamin is necessary for the production of the normal physiological actions of the corticosteroids, and such a function

TABLE 4. *Adrenal ascorbic acid depletion in assay rats induced by injection of (a) ACTH and (b) plasma (2 ml/100 g) from guinea-pigs on a normal diet or a diet deficient in ascorbic acid*

ACTH injected (mμ/100 g body weight)	Adrenal ascorbic acid depletion (mg/100 g adrenal tissue ± S.E.)
0·0625	18 ± 5·1
0·25	40 ± 3·8
1·0	72 ± 6·8
4·0	100 ± 4·9
16·0	127 ± 3·5

(a)

Days on ascorbic acid deficient diet	Treatment	Adrenal ascorbic acid depletion (mg/100 g adrenal tissue ± S.E.)	Plasma ACTH concentration (mU/100 ml)
0	None	30 ± 4·2	6·6
14	None	31 ± 4·0	7·0
21	None	104 ± 3·6	245·5
21	Betamethasone 20 μg/ml throughout	6 ± 3·7	Not detectable
21	Ascorbic acid 1 g intraperitoneally 90 min before collection of plasma	22 ± 3·4	< 5·0
21	Plasma incubated for 24 h at 37° C	8 ± 2·8	Not detectable

(b)

for ascorbic acid is not incompatible with our data. The enormously increased activity of the hypothalamo-pituitary-adrenocortical system which occurs in the terminal stages of scurvy is not due simply to the non-specific stress of ascorbic acid deficiency because, although it can be inhibited by betamethasone, the steroid is ineffective in prolonging the lives of the scorbutic animals. Our observations confirm the lack of involvement of ascorbic acid in corticoidogenesis but do not preclude the possibility that it is necessary for the proper utilization of corticoids by the tissues.

The work was financed by a generous grant from Beecham Research Laboratories to whom we are very grateful. Our thanks are also due to Mr. E. Vowles for invaluable technical assistance.

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(*Received April* 19, 1971)