Br. J. Pharmac. (1970), **40**, 740–746.

Ascorbic acid deficiency and pituitary adrenocortical activity in the guinea-pig

J. R. HODGES AND R. T. HOTSTON

Department of Pharmacology, Royal Free Hospital School of Medicine, London W.C.1

Summary

1. Guinea-pigs kept on a diet deficient in ascorbic acid lost weight and became moribund in about 24 days.

2. The adrenal ascorbic acid concentration fell rapidly during the first 2 weeks, and the plasma corticosteroid concentration and 17-oxogenic steroid excretion rose sharply in the third week of ascorbic acid deficiency.

3. Both histamine and corticotrophin increased the plasma corticosteroid concentration when injected during the second week but failed to change the pre-existing high concentration of the steroid in the third week of ascorbic acid deficiency.

4. The observations confirm that ascorbic acid is not involved in corticoidogenesis and that scurvy is a severe stress which increases adrenocortical activity to such an extent that the rate of synthesis of corticosteroids is incapable of matching the rate of their release.

Introduction

Ascorbic acid, normally present in adrenal glands in high concentrations, is usually depleted by exposure to stress or by the injection of corticotrophin (ACTH). Although this fall in adrenal ascorbic acid concentration is associated with increased adrenocortical activity, the precise function of the vitamin in the synthesis and release of corticosteroids is not understood. The concentration of ascorbic acid in the adrenal glands of the guinea-pig can be reduced rapidly to scarcely detectable levels by diets deficient in ascorbic acid (Oesterling & Long, 1951) and this paper describes the results of experiments in which pituitary adrenocortical activity was studied in guinea-pigs made scorbutic in this way.

Methods

Animals. One hundred and forty female Dunkin Hartley guinea-pigs (Beecham Research Laboratories), weighing between 250 and 350 g, were used. They were fed on rat cubes (Diet 41B, Lane-Petter & Dyer, 1952) which contain no vitamin C, and tap water *ad lib.* For 10 days the diet was supplemented with greenfood and only those animals which grew satisfactorily were used. Greenfood was then withdrawn and the animals were divided into test and control groups. The test group received no ascorbic acid and the controls the same diet with ascorbic acid (0·2 mg/ml) in the drinking water. This concentration was selected after preliminary experiments on fluid intake to ensure that every animal received approximately 10 mg

ascorbic acid/day. Blood and urine samples and adrenal glands were collected at various times during the course of the experiment and, in some cases, 2 h after the injection of corticotrophin or exposure to stress (injection of histamine).

Blood samples. The animals were anaesthetized with 25% (w/v) urethane solution (0·5 ml/100 g body weight) and blood was collected from a large mesenteric artery into 10 ml glass syringes. The samples were collected within 2 min of removal of the animals from their cages, centrifuged within 1 h of collection, and the plasma was stored at −12° C. Corticosteroid concentrations were measured by the method of Zenker & Bernstein (1958) and the results expressed as cortisol and corticosterone calculated as described by Stockham (1963).

Adrenal glands. Adrenal glands were removed immediately after the withdrawal of the blood samples, dissected free from peri-adrenal fat and connective tissue and weighed on a torsion balance. Left adrenals were ground with the aid of sand and a glass rod in 4% (w/v) trichloracetic acid solution and their ascorbic acid contents were determined by the method of Roe & Kuether (1943). Right adrenals were ground similarly in 10% (v/v) ethanol for the determination of cortisol and corticosterone.

Urine. Twenty-four hour urine samples were collected using conventional metabolism cages, and frozen until analysis for 17-oxo and 17-oxogenic steroids as described by Besch & Barry (1964).

Drugs. Histamine acid phosphate 2·5 mg/ml and corticotrophin (Cortrophin, Organon) 5 i.u./ml in normal saline were injected intraperitoneally and subcutaneously, respectively, in volumes of 0·1 ml/100 g body weight.

Results

The growth rate of the guinea-pigs is shown in Fig. 1. The test animals continued to grow well until the thirteenth day on the ascorbic acid deficient diet, when they

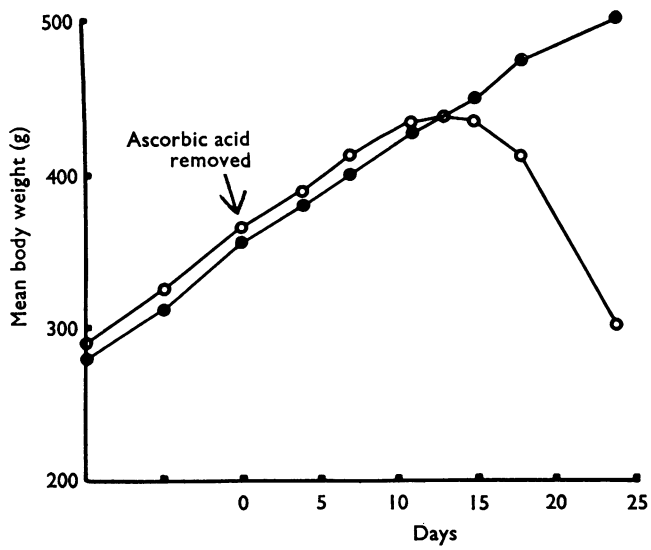

FIG. 1. Growth rate in guinea-pigs on a diet deficient in ascorbic acid. Controls ●——●; tests ○——○.

began to lose weight markedly. After 24 days they were moribund. Control animals grew normally. The adrenal ascorbic acid concentration fell rapidly during the first week of vitamin C deficiency and then more slowly. After 2 weeks the adrenals were almost completely depleted of the vitamin and by the third week there was none present. The adrenal ascorbic acid content in the control animals did not alter (Fig. 2). The ascorbic acid deficiency caused adrenal hypertrophy and at the end of the experiment the adrenal weights of the test animals were significantly ($P<0.05$) greater than those of the controls (Table 1).

The plasma cortisol and corticosterone concentrations in the test animals did not differ from the controls for the first 17 days despite the almost complete disappearance of ascorbic acid from the adrenal glands. Subsequently, they rose very rapidly and by the end of the experiment were almost 10 times those of the controls in which the plasma corticosteroid concentrations remained unchanged (Fig. 3).

Adrenal cortisol and corticosterone concentrations in the test animals remained constant for the first 17 days (Fig. 4), after which they increased considerably, reached a maximum on the twentieth day and then fell sharply. By the end of the experiment the adrenal corticosteroid concentrations were well below their initial values.

TABLE 1. *Effect of ascorbic acid deficiency on adrenal weight in the guinea-pig*

Day of experiment	Mean adrenal weight (mg±standard error)		Mean greatest body weight (g)		Ratio of adrenal weight (mg) to greatest body weight (g)	
	Control	Test	Control	Test	Control	Test
0	111·6±7·0		366		0·305	
7		126·3±6·6		383		0·329
17	162·3±12·6	165·3±14·0	447	472	0·362	0·344
24	206·5±12·6	265·6±18·1	526	477	0·392	0·557

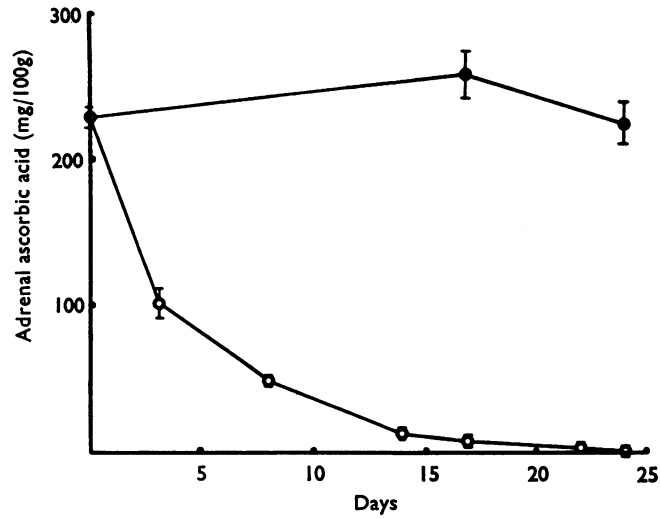

FIG. 2. Adrenal ascorbic acid concentrations in guinea-pigs on a diet deficient in vitamin C. Controls ●——●; tests ○——○. Vertical bars indicate standard errors.

Ascorbic acid deficiency caused an increase in 17-oxo steroid excretion which reached a peak on the fourteenth day and remained elevated for the next 7 days. In contrast, the excretion of 17-oxogenic steroids fell during the first 2 weeks of ascorbic acid deficiency and then increased considerably (Fig. 5).

Control animals responded to the injection of corticotrophin or histamine with a considerable rise in the plasma cortisol concentration (Fig. 6). The responses to

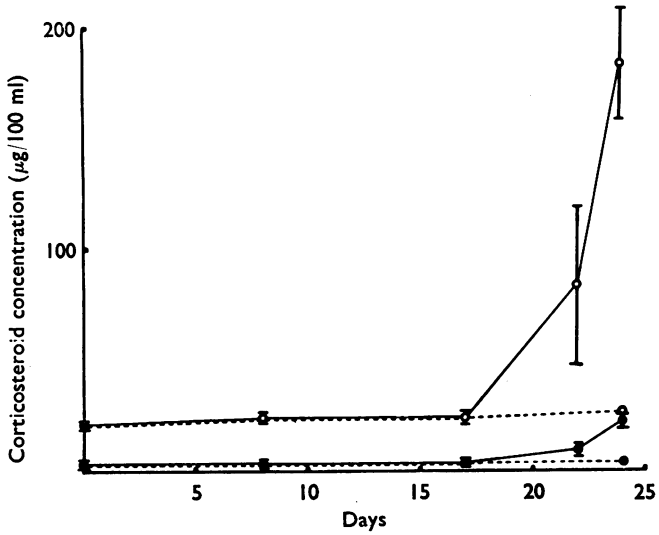

FIG. 3. Plasma corticosteroid concentrations in guinea-pigs on a diet deficient in ascorbic acid. ○——○, Cortisol; ●——●, corticosterone concentrations. The dotted lines show control values and the vertical bars the standard errors.

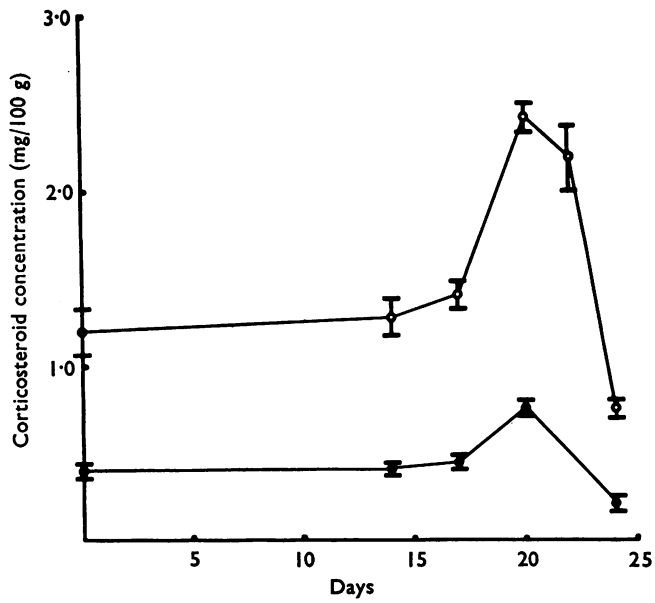

FIG. 4. Adrenal cortisol (○——○) and corticosterone (●——●) concentrations in guinea-pigs on a diet deficient in ascorbic acid. The vertical bars indicate the standard errors.

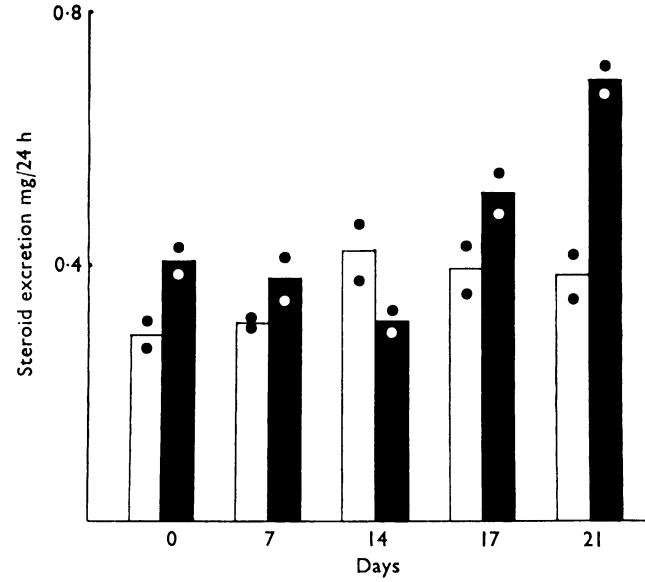

FIG. 5. Urinary 17-oxo (□), and 17-oxogenic (■) steroids excreted in 24 h by guinea-pigs on a diet deficient in ascorbic acid. The dots indicate individual values.

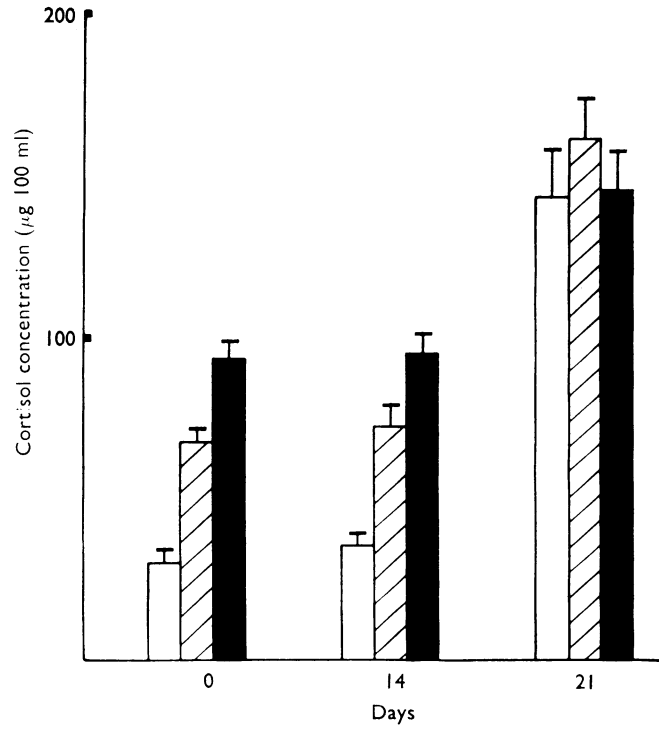

FIG. 6. Plasma cortisol concentrations 2 h after intraperitoneal injection of 0·1 ml/100 g saline (□), 250 μg/100 g histamine (▨), and subcutaneous injection of 0·5 i.u./100 g ACTH (■), in guinea-pigs on a diet deficient in ascorbic acid. The vertical bars indicate the standard errors.

these stimuli were not different after 2 weeks on the diet deficient in ascorbic acid. However, after 3 weeks of ascorbic acid deficiency, the pre-existing high plasma corticoid concentrations were not increased further by the administration of either histamine or corticotrophin.

Discussion

Although it is more than 40 years since the presence of ascorbic acid in adrenal glands was first demonstrated by Szent-Gyorgyi (1928), the functional importance of the vitamin in corticosteroid synthesis and release is still not known.

Our work does not support the view (Giroud, Martinet & Bellon, 1941 ; Lockwood & Hartman, 1933 ; Lowenstein & Zwemer, 1946) that ascorbic acid is necessary for the synthesis of corticosteroids. Guinea-pigs with virtually no ascorbic acid in their adrenal glands responded to stress or the injection of corticotrophin with normal changes in plasma corticosteroid concentration. The remarkably high blood levels of cortisol and corticosterone which occurred in the later stages of scurvy also suggest that corticoidogenesis can proceed in the absence of ascorbic acid. However, the elevated plasma corticosteroid concentrations in the scorbutic animals need not necessarily have been due to increased adrenocortical secretion but to impaired steroid metabolism and excretion. Such a possibility is not likely because ascorbic acid deficiency causes no change in the clearance or conjugation of exogenous cortisol (Jones, Peric-Golia & Eik-Nes, 1958) and there is a parallelism between the plasma cortisol concentration and 17-oxogenic steroid excretion in the second and third week of ascorbic acid deficiency. The initial drop in 17-oxogenic steroid excretion is not easy to explain, but a similar fall followed by a rapid rise was also seen in ascorbic acid deficient guinea-pigs by Prunty, Clayton, McSwiney & Mills (1955). However, we were unable to confirm their demonstration of a fall in 17-oxo steroid excretion in the second week of vitamin C deficiency, by which time 17-oxo steroid excretion in our animals had reached its peak.

Again our findings are not in accord with the possibility that ascorbic acid exerts an inhibitory effect on corticoidogenesis as has often been suggested (Hayano, Saba, Dorfman & Hechter, 1956 ; Jones *et al.*, 1958 ; Kitabchi, 1967). Kapanowski (1969) observed that animals with ascorbic acid deficiency show augmented adrenocortical responses to corticotrophin, but we were unable to confirm his work. Furthermore, although marked changes in plasma corticosteroid concentrations and urinary 17-oxogenic steroid excretion occurred in our scorbutic guinea-pigs, the considerable time lag between the fall in adrenal ascorbic acid and the increase in adrenocortical activity makes involvement of ascorbic acid in the inhibition of adrenal corticosteroidogenesis unlikely.

It appears that ascorbic acid is not essential for the elaboration of corticosteroids (Eisenstein & Shank, 1951) and that the increased adrenocortical activity in the terminal stages of scurvy is caused by the severe stress imposed by vitamin C deficiency (Howard & Cater, 1959 ; Oesterling & Long, 1951). A drop in adrenal corticosteroid concentration occurs in the final stages of ascorbic acid deficiency, possibly because the rate of synthesis of the corticosteroids is incapable of matching the rate of their release. The inability of stress or ACTH to provide a further increase in the already very high plasma cortisol concentration also suggests that the adrenal glands are under maximal stimulation by endogenous ACTH. Direct

estimation of circulating corticotrophin in scorbutic animals may provide more information on the role of ascorbic acid in pituitary-adrenocortical physiology.

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.

REFERENCES

BESCH, P. K. & BARRY, R. D. (1964). In: *Steroid Determinations*, pp. 28–31; 41–45. Ohio: American Medical Technologists.

EISENSTEIN, A. B. & SHANK, R. E. (1951). Relationship of ascorbic acid to secretion of adrenocortical hormones in guinea-pigs. *Proc. Soc. exp. Biol. Med.*, **78**, 619–624.

GIROUD, A., MARTINET, M. & BELLON, M. T. (1941). Dépendance de la fonction cortico-surrénale, chez l'homme, vis-a-vis de l'acide ascorbique: Variations de l'élimination urinaire de l'homme corticale. *C. r. Séanc. Soc. Biol.*, **135**, 514–516.

HAYANO, M., SABA, N., DORFMAN, R. I. & HECHTER, O. (1956). Some aspects of the biogenesis of adrenal steroid hormones. *Recent Prog. Horm. Res.*, **12**, 79–123.

HOWARD, A. N. & CATER, D. B. (1959). The adrenal cortex and the effect of ACTH and cortisone in scorbutic guinea-pigs. *J. Endocr.*, **18**, 175–185.

JONES, R. S., PERIC-GOLIA, L. & EIK-NES, K. (1958). Ascorbic acid deficiency and adrenocortical function in the guinea-pig. *Endocrinology*, **63**, 659–668.

KAPANOWSKI, L. (1969). Evaluation of adrenal function in experimental vitamin C deficiency. *Acta. med. pol.*, **10**, 119–136.

KITABCHI, A. E. (1967). Ascorbic acid in steroidogenesis. *Nature, Lond.*, **215**, 1385–1386.

LANE-PETTER, W. & DYER, F. J. (1952). *Technical note No.* 7: *Compressed Diets.* London: Laboratory Animals Bureau.

LOCKWOOD, J. E. & HARTMAN, F. A. (1933). Relation of adrenal cortex to vitamins A, B_1 & C. *Endocrinology*, **17**, 501–521.

LOWENSTEIN, B. E. & ZWEMER, R. L. (1946). The isolation of a new active steroid from the adrenal cortex. *Endocrinology*, **39**, 63–64.

OESTERLING, J. & LONG, C. N. H. (1951). Adrenal cholesteral in the scorbutic guinea-pig. *Science, N.Y.*, **113**, 241–242.

PRUNTY, M. A., CLAYTON, B. E., MCSWINEY, R. R. & MILLS, I. H. (1955). Studies of the interrelationship between the adrenal cortex and ascorbic acid metabolism. *Ciba Fdn Colloq. Endocr.*, **8**, 324–339.

ROE, J. H. & KUETHER, C. A. (1943). The determination of ascorbic acid in whole blood and urine, through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *J. biol. Chem.*, **147**, 399–407.

STOCKHAM, M. A. (1963). The assay of cortisol and corticosterone in human plasma. *J. Endocr.* **26**, IV–V.

SZENT-GYORGYI, A. J. (1928). Observations of the function of peroxidase systems and the chemistry of the adrenal cortex: Description of a new carbohydrate derivative. *Biochem. J.*, **22**, 1387–1409.

ZENKER, N. & BERNSTEIN, D. E. (1958). The estimation of small amounts of corticosterone in rat plasma. *J. biol. Chem.*, **231**, 695–701.

(*Received August* 19, 1970)