REFERENCES

Balch, C. C. (1950). *Brit. J. Nutr.* **4**, 361.
Balch, C. C., Balch, D. A., Bartlett, S., Johnson, V. W., Rowland, S. J. & Turner, J. (1954). *J. Dairy Res.* **21**, 305.
Blaxter, K. L. (1944). *Proc. Brit. Soc. Anim. Prod.* 2nd Mtg, p. 85.
Blaxter, K. L. (1950–1). *Nutr. Abstr. Rev.* **20**, 1.
Blaxter, K. L. & Graham, N. McC. (1955*a*). *J. agric. Sci.* **46**, 292.
Blaxter, K. L. & Graham, N. McC. (1955*b*). *Proc. Nutr. Soc.* **14**, xv.
Blaxter, K. L., Graham, N. McC. & Wainman, F. W. (1956). *Proc. Nutr. Soc.* **15**. (In the Press.)
Bohstedt, G., Roche, B. H., Rupel, I. W. & Fuller, J. G. (1930). *Bull. Univ. Wis. Sci. Ser.* no. 10.
Cole, H. H. & Mead, S. W. (1943). *Science*, **98**, 543.
Ewing, P. V. & Smith, F. H. (1917). *J. agric. Res.* **10**, 2.
Forbes, E. B., Fries, J. A. & Braman, W. W. (1925). *J. agric. Res.* **31**, 987.
Heller, V. G., Wall, R. & Briggs, H. M. (1911). *Tech. Bull. Okla. agric. Exp. Sta.* no. 10.
Kick, C. H., Gerlaugh, P., Schalk, A. F. & Silver, A. (1937). *J. agric. Res.* **55**, 587.
Lehmann, F. (1941). *Z. Tierernähr.* **5**, 155.
Lenkeit, W. (1930). *Arch. Tierernähr. Tierz.* **2**, 626.
Mead, S. W. & Goss, H. (1935). *J. Dairy Sci.* **18**, 163.
Phillipson, A. T. (1952). *J. Physiol.* **116**, 84.
Piana, C. (1952). *Zootec. e Vet.* **11**, 12.
Raymond, W. F., Harris, C. E. & Harker, V. G. (1953). *J. Brit. Grassl. Soc.* **8**, 301.
Schneider, B. H. (1954). In *Festschrift annlässlich des 100 jährigen Bestehens der Landwirtschaftlichen Versuchsstation Leipzig-Möckern. Vol. 2. 100 Jahre Möckern. Die Bewertung der Futterstoffe und andere Probleme der Tierernährung*, p. 233. [K. Nehring, editor.] Berlin: Deutscher Bauernverlag.
Swanson, E. W. & Herman, H. A. (1952). *J. Anim. Sci.* **11**, 692.
Usuelli, F. (1933). *Profilassi*, **6**, 7.

Effect of continued administration of cortisone and ACTH on vitamin C levels in guinea-pig adrenals*

By B. J. CONSTABLE, LESLIE J. HARRIS and R. E. HUGHES

Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council

(*Received* 23 *July* 1955)

It is well known that an injection of ACTH gives rise to a transient fall in the concentration of adrenal ascorbic acid (Sayers, Sayers, Lewis & Long, 1944); this action forms the basis for a standard assay procedure in which the hormone is administered to hypophysectomized rats (Sayers & Sayers, 1946). Since one important effect of ACTH is to stimulate the production of corticosteroids by the adrenals, it may be asked whether cortisone itself can be shown to have any corresponding action on vitamin C levels, in the adrenals or in other tissues. It was to investigate this question that the experiments to be described were conducted. We have previously found that one common effect of cortisone with ACTH was that, when repeatedly injected, it brought about the remarkable increase in liver weight observed in guinea-pigs and in rabbits (Hughes, Harris, Constable & Bland, 1952; Bland, Constable, Harris &

* Read in part before The Nutrition Society on 7 May 1955 (Constable, Harris & Hughes, 1955).

Hughes, 1952*a*, *b*; Harris, Bland, Hughes & Constable, 1953; Cherry, Bland, Constable, Hughes & Harris, 1954).

The results now to be recorded indicate that the continued administration of cortisone to guinea-pigs kept on known intakes of vitamin C did in fact cause a pronounced fall in the concentrations of vitamin C in their adrenals. The effect therefore resembles that previously reported in guinea-pigs for long-term injections of ACTH (Harris *et al.* 1953). It should be noted that in our tests the guinea-pigs were not subjected to hypophysectomy and were maintained on controlled levels of vitamin C intake for the duration of the experiment.

EXPERIMENTAL

General procedure. Three separate experiments were done with cortisone, the first involving seventy guinea-pigs, the second fifty and the third forty. A parallel test was done also with ACTH.

Table 1. *Experimental diet used in preliminary period*

Ingredient	Parts by weight
Sussex-ground oats	57·5
Bran	13·0
Dried skim milk (roller-dried)	21·5
Dried yeast	8·0
Salt mixture	2·0
Supplements:	
Daily: cabbage (g)	15
Weekly: vitamin E (α-tocopheryl acetate, 5% in arachis oil) (drops)	1
vitamin K (2-methyl-1:4-naphthoquinone, 5% in arachis oil) (drops)	1
halibut-liver oil (drops)	1

In all experiments, young male guinea-pigs, weighing about 250 g, were placed for a preliminary period of about 14 days on the standard basal diet (Table 1) supplemented with cabbage (15 g daily) and received water without stint.

When the guinea-pigs reached 300 g in weight, for some the cabbage was discontinued and various graded allowances of ascorbic acid (ranging from 0 to 50 mg daily) were administered instead, as described below; other guinea-pigs received cabbage throughout. The ascorbic acid was given orally in aqueous 1% (w/v) citric-acid solution. For every such level of ascorbic acid, one set of guinea-pigs was injected intramuscularly twice daily, at 9 a.m. and 5 p.m., with cortisone (administered as the acetate), and a corresponding set was kept as control, receiving instead a dummy injection of the aqueous vehicle used for making up the cortisone (Aqueous Vehicle No. 1, Merck and Co., Inc., Rahway, New Jersey, U.S.A.). The injections were continued for either 10 or 14 days, as indicated later, and then the animals were killed for an immediate chemical analysis and for pathological studies. No injections were given on the morning of the day when the animals were to be killed, to minimize any merely transient effect caused by a single injection.

For the experiments with ACTH the same procedure was followed, except that ACTH and the corresponding dummy injections (normal saline in this instance) were substituted for cortisone and its dummy.

Experiment 1. The five levels of ascorbic-acid administration were 0, 0·5, 1·0, 2·0 and 4·0 mg daily; the dose of cortisone was 7·5 mg daily; the experimental period during which both the vitamin C administration and the cortisone, or dummy, injections were maintained was 14 days; and there were twelve animals in each group (i.e. six cortisone-injected and six dummy controls), making sixty in all. An additional group of ten guinea-pigs (five injected with cortisone and five with dummy) received cabbage, 15 g/day, in place of pure ascorbic acid.

Experiment 2. The plan differed slightly from that of Exp. 1 in that during the first 10 days graded doses of ascorbic acid were given (0, 0·5, 1·0, 2·0 and 5·0 mg), but without any injections, and that during the next 10 days this treatment was continued with the addition of daily injections of cortisone (twice as much as in Exp. 1, namely, 15 mg daily) or of the dummy. There were ten animals in each group (five injected with the cortisone and five with the dummy control).

Experiment 3. The experimental details were as in Exp. 2, except that the levels of ascorbic acid were 2, 10 and 50 mg. A further group of ten guinea-pigs (five cortisone-injected and five dummy controls) had cabbage, 15 g/day, in place of ascorbic acid.

Experiment 4. In this experiment, ACTH was used in place of cortisone. The levels of ascorbic acid were 0, 0·5, 1·0, 2·0, 5·0 and 10·0 mg daily, continued for 20 days. During the second half of the 20-day period, ACTH (L.A. 1.A., Armour Laboratories, Armour and Co., Chicago, Ill., U.S.A.) was injected at the rate of 8 mg daily in four equal doses, at 9 a.m., 1 p.m., 5 p.m. and 9 p.m. There were ten guinea-pigs in each of the six groups, five being injected with ACTH and the remaining five with the dummy.

RESULTS

Effect of cortisone on adrenal glands

In Figs. 1–3 are shown the effects (at each level of vitamin C intake) of the cortisone injections upon (1) the adrenal weights, (2) the adrenal ascorbic-acid concentrations and (3) the total quantity of ascorbic acid in the adrenals. (These figures apply to the groups in which ascorbic acid was given as such; the results with cabbage in the diet were essentially similar and will be considered separately below.)

Weight. In all three experiments, the adrenal weights were notably depressed in the cortisone-injected animals as compared with the controls (Fig. 1). The effect was greater with 15 mg than with 7·5 mg. In explanation of this diminished size, it may be supposed that the adrenal does not have to work so hard when its natural secretion, or some portion thereof, is supplied ready-made from outside (see Ingle & Kendall, 1937; Ingle & Mason, 1938); alternatively, the effect of the injected steroid could be regarded as causing a fall in ACTH output, and hence a diminution in adrenal activity (see Long, 1952).

Ascorbic-acid concentration. As was to be expected, there was more ascorbic acid in the adrenals of the animals on the high intakes of vitamin C than of those on the

lower intakes (Fig. 2). The effect of the cortisone, when given at the rate of 15 mg daily, was to depress the concentration of ascorbic acid as compared with the controls at each such level of intake. This depression, when expressed on a percentage basis, was sometimes most marked with the lower intakes of vitamin C, when the concentration in the non-injected, control animals was itself low. Indeed, with the smaller dose of cortisone, 7·5 mg, the effect was seen only at the three lower levels of intake, and not at the two higher ones; it occurred, nevertheless, in the group receiving cabbage, supplying a high intake of vitamin C (see below).

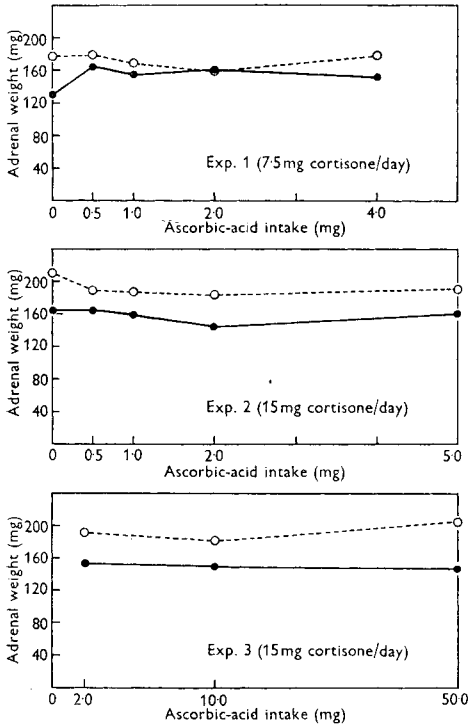

Fig. 1

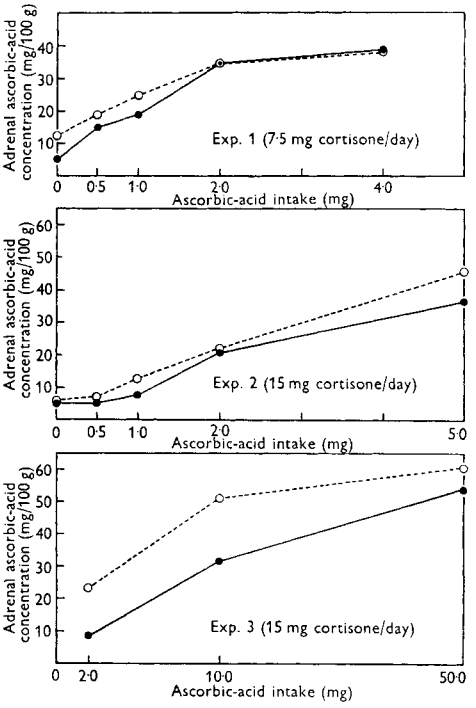

Fig. 2

Fig. 1. Exp. 1 (six animals/set), Exps. 2 and 3 (five animals/set). Adrenal weights in guinea-pigs kept on graded intakes of vitamin C, as influenced by continued injections of cortisone. ——, cortisone-treated animals; - - - -, dummy controls.

Fig. 2. Exp. 1 (six animals/set), Exps. 2 and 3 (five animals/set). Effect of cortisone on concentration of ascorbic acid in guinea-pigs' adrenals at different levels of vitamin intake. ——, cortisone-treated animals; - - - -, dummy controls.

Ascorbic-acid content. Expressed on the basis of the total weight of ascorbic acid in the adrenals (Fig. 3), the influence of cortisone was even more obvious, since not only were the concentrations of the ascorbic acid diminished, but the weights of the adrenals themselves were less.

'*Cabbage*' *groups.* In the two groups of animals receiving cabbage, 15 g/day, in place of synthetic ascorbic acid, the same trends were apparent. That is to say, there occurred, in the cortisone-injected animals, compared with the controls injected with the dummy, a fall in adrenal weight, and a fall in the adrenal ascorbic acid, expressed

either as concentration or as total amount. This fall was seen at both levels of injection of cortisone, 7·5 and 15 mg. The results for these 'cabbage' groups are, for convenience, not included in Figs. 1–3, but will be found instead in Table 2.

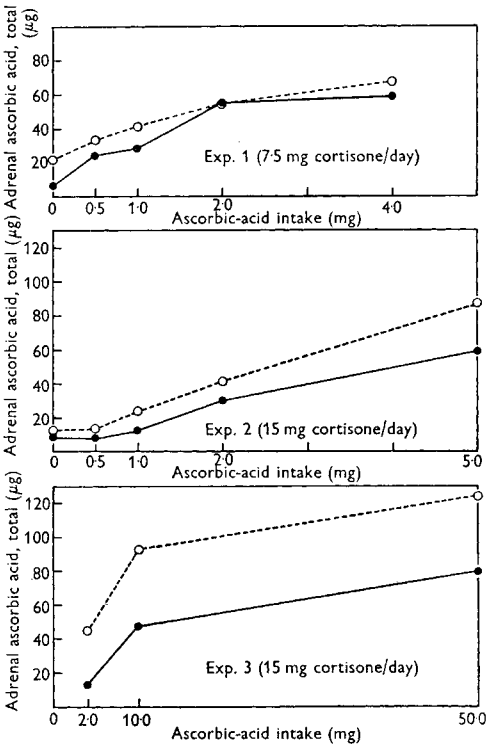

Fig. 3. Exp. 1 (six animals/set), Exps. 2 and 3 (five animals/set). Effect of cortisone on total ascorbic acid in guinea-pigs' adrenals. ——, cortisone-treated animals; - - - -, dummy controls.

Table 2. *Effect of cortisone on adrenal weights and adrenal ascorbic acid in guinea-pigs receiving 15 g cabbage daily in addition to the experimental diet. (Mean values for five animals)*

Exp. no.	Cortisone (mg/day)	Adrenal weight (mg)	Adrenal ascorbic acid: Concentration (mg/100 g)	Adrenal ascorbic acid: Total (μg)
1	0	170	136	231
	7·5	161	111	179
3	0	205	106	217
	15	179	96	172

Reproducibility of results. As already indicated, the effect of the cortisone, when given at the higher dose (15 mg), was to produce the same result (a fall in adrenal vitamin C concentration) repeatedly and consistently, in both experiments and in all eight groups of animals, and at all the varying levels of ascorbic-acid intake tested, ranging from 0 to 50 mg daily. (Similarly, when the smaller dose of cortisone was given, this same depressing effect was still noted consistently at the three lower levels of vitamin C intake, although it was not apparent at the two top levels of intake.) In

Table 3. *Evaluation of statistical significance of findings in experiments (Exps. 1, 2 and 3) on the effect of cortisone on ascorbic acid in guinea-pig adrenals*

Exp. no.	No. of animals in each set	Treatment: Cortisone (mg/day)	Treatment: Vitamin C (mg/day)	Effect observed: Mean decrease in adrenal weight, as percentage of control	Effect observed: Mean decrease in adrenal ascorbic-acid concentration, as percentage of control	Effect observed: Mean decrease in total adrenal ascorbic acid, as percentage of control	'Combination of probabilities' test (Fisher, 1954). Values of χ^2, with significance ratings: Decrease in adrenal weight	Decrease in adrenal ascorbic-acid concentration	Decrease in total adrenal ascorbic acid
1	6	7·5	0	26·5 ± 7·99	59·0 ± 15·00	69·2 ± 16·02			
			0·5	7·7 ± 8·10	20·8 ± 7·81	25·9 ± 9·62			
			1·0	8·4 ± 8·86	24·7 ± 8·06	31·5 ± 11·55			
			2·0	−1·9 ± 6·33	0·3 ± 12·29	0·7 ± 14·84	27·01**	40·18***	43·30***
			4·0	14·7 ± 8·75	−1·8 ± 13·01	10·7 ± 17·30			
			C†	5·3 ± 8·27	18·4 ± 11·63	22·3 ± 11·02			
2	5	15·0	0	20·8 ± 8·74	13·2 ± 39·74	27·5 ± 36·10			
			0·5	12·7 ± 8·30	28·5 ± 17·31	36·6 ± 29·59			
			1·0	15·0 ± 9·02	39·1 ± 18·61	44·8 ± 18·73	32·06***	20·80*	25·47**
			2·0	21·3 ± 8·04	5·8 ± 11·83	27·1 ± 29·03			
			5·0	15·3 ± 8·46	19·6 ± 14·31	31·7 ± 13·58			
3	5	15·0	2·0	19·9 ± 8·77	63·1 ± 17·24	71·9 ± 23·69			
			10·0	17·2 ± 5·19	38·1 ± 9·08	49·0 ± 10·16	43·68***	30·46***	40·04***
			50·0	28·2 ± 4·26	10·7 ± 13·80	35·2 ± 16·09			
			C†	12·7 ± 5·35	9·4 ± 11·49	20·6 ± 7·78			
1, 2, 3	Aggregate evaluation						102·74***	91·44***	108·81***

*** denotes $P < 0·001$. ** denotes $P = 0·01–0·001$. * denotes $P = 0·05–0·01$.

† C = intake of vitamin C from 15 g cabbage.

Table 4. *Evaluation of statistical significance of findings in experiments (Exp. 4, five animals in each set) on the effect of ACTH on ascorbic acid in guinea-pig adrenals*

Treatment: ACTH (mg/day)	Treatment: Vitamin C (mg/day)	Effect observed: Mean increase in adrenal weight, as percentage of control	Effect observed: Mean decrease in adrenal ascorbic-acid concentration, as percentage of control	Effect observed: Mean increase in total adrenal ascorbic acid, as percentage of control	'Combination of probabilities' test (Fisher, 1954). Values of χ^2, with significance ratings: Increase in adrenal weight	Decrease in adrenal ascorbic-acid concentration	Increase in total adrenal ascorbic acid
8	0	50·6 ± 14·53	11·3 ± 34·30	39·8 ± 40·67			
	0·5	62·5 ± 8·91	14·0 ± 20·49	42·3 ± 27·56			
	1·0	99·3 ± 25·42	25·9 ± 10·23	42·0 ± 16·27	90·71***	29·25**	44·25***
	2·0	91·6 ± 9·22	31·8 ± 10·83	31·8 ± 23·71			
	5·0	91·5 ± 15·16	−25·4 ± 14·73	139·8 ± 25·03			
	10·0	70·6 ± 14·71	20·3 ± 10·90	38·0 ± 20·05			

*** denotes $P < 0·001$. ** denotes $P = 0·01–0·001$.

the two groups receiving cabbage in place of ascorbic acid the result was again a fall in adrenal ascorbic acid. From these results as a whole, therefore, there seems no doubt about the reality of the phenomenon.

Statistical analysis. Dr J. Wishart has kindly submitted our results to statistical analysis, and his report is as follows: 'The mean decreases which are at least $2\frac{1}{4}$–$2\frac{1}{2}$

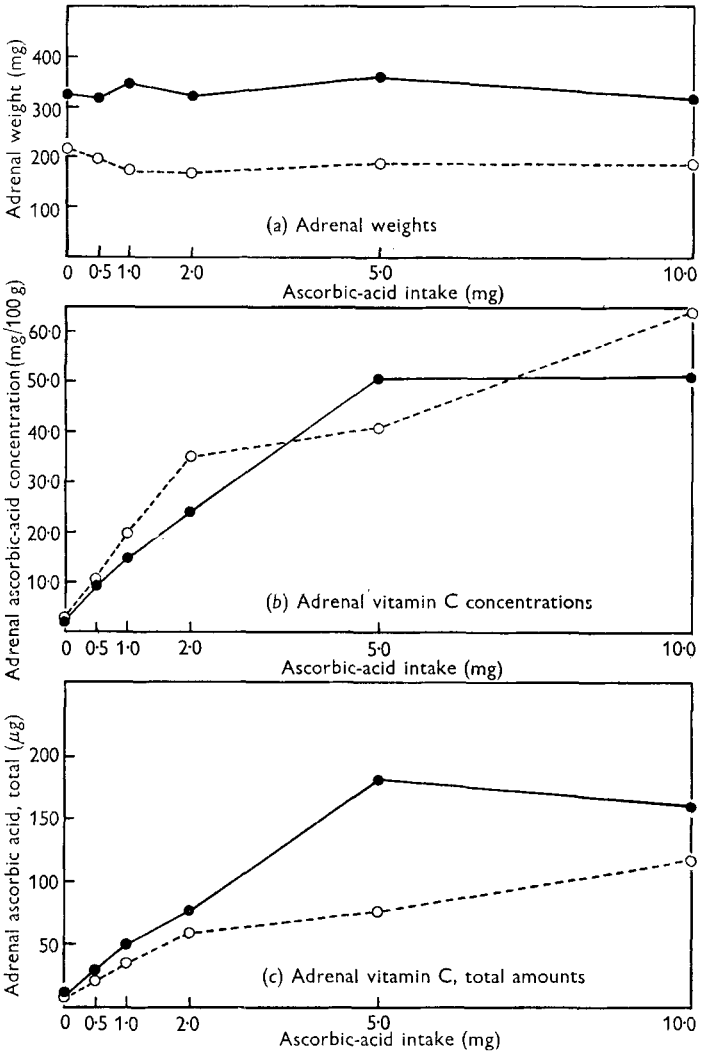

Fig. 4. Exp. 4 (five animals/set). Adrenal weights and adrenal ascorbic acid (concentration and total) of guinea-pigs, as influenced by continued injection of ACTH (8 mg/day). ——, ACTH-treated animals; - - - -, dummy controls.

times their standard errors, depending upon the sizes of the samples compared, are significant. This accounts for some 40% of the results in Table 3, where the mean decreases are given together with their standard errors. The remainder, although not individually significant, are in the same direction (i.e. decreases), with the exception of two small mean increases. This makes it appropriate to summarize the results by

means of the "combination of probabilities" test (Fisher, 1954). This takes into account the sign of the mean decrease, and is expressed as a value of χ^2 having for degrees of freedom twice the number of tests included. These values are given in Table 3 with an indication of the level of significance attained, i.e. what is there called the "significance rating". The results are all significant for the three separate experiments, and very much so for the three experiments combined. This shows that the three effects produced by the cortisone, namely, the fall in adrenal weight, the fall in adrenal ascorbic-acid concentration and the fall in total adrenal ascorbic acid, are most unlikely to be due to chance.'

Effect of ACTH on adrenal glands

The ACTH had an influence similar to that of cortisone in diminishing the adrenal ascorbic acid, when this effect is expressed in terms of concentration. This is seen at five out of the six levels of vitamin C intake tested (Fig. 4). However, because of the great enlargement of the adrenals caused by the ACTH, the ascorbic-acid content, when expressed not as a concentration but as the total amount in the glands, so far from being diminished, was in fact increased (Fig. 4).

Statistical significance. Dr Wishart reports as follows: 'Of these mean increases and decreases half are significant, and of the remainder only one is in the unexpected direction (shown as negative, Table 4). A further application of the "combination of probabilities" test shows significant results for all three effects, so that we can conclude that the increases in adrenal weight and in total adrenal ascorbic acid, and the decrease in adrenal ascorbic-acid concentration, are very unlikely to be chance results.'

Effect of cortisone and ACTH on adrenal weight in relation to the vitamin C intake

The decrease in adrenal weight with cortisone (Fig. 1) and the increase with ACTH (Fig. 4, top panel) did not depend on the level of intake of vitamin C, under the conditions of our tests, which is at once apparent from an inspection of the graphs. This finding will be referred to again below.

DISCUSSION

The principal new finding that emerges from these investigations is that cortisone, when administered over a period of time, brings about a fall in ascorbic acid in the adrenal glands. This effect may be compared with the purely transient drop in adrenal ascorbic acid caused by a single dose of ACTH, as observed in hypophysectomized rats in the well-known Sayers test (Sayers & Sayers, 1946). It may be noted, however, that in our own experiments the conditions were different in many ways and had some novel features. For example, guinea-pigs and not rats were used; the effect was a long-term one; there was no hypophysectomy; and the results were checked at various graded levels of vitamin C intake. It may be noted, nevertheless, that long-term administration of ACTH to guinea-pigs can likewise cause a diminution in adrenal ascorbic-acid concentration, as previously recorded by us (Harris *et al.* 1953). This finding was examined in further detail in the experiments reported here.

The real explanation of this diminution in adrenal ascorbic acid is still obscure. Our object here has been to present a factual summary of our main experimental findings, rather than to embark on speculations about their possible implications or their theoretical foundation, and we believe that that would probably in any event be premature at the present stage of knowledge. It is worth pointing out, however, that, although cortisone brings about this localized fall in the vitamin C in the adrenals, it does not, in our experience, measurably influence the vitamin C requirements of the organism as a whole (Harris, Constable, Hughes & Loewi, 1955*a*, *b*).

Another of our findings was that neither the decrease in adrenal weight produced by cortisone nor the increase by ACTH was influenced by the level of intake of vitamin C, under our experimental conditions. This result may be considered in conjunction with the fact that a well-known, and much discussed, feature of scurvy in guinea-pigs is the abnormal appearance of the adrenals, which is accompanied by an increase in their volume. This effect has sometimes been held to denote an intimate interrelation between vitamin C and adrenal function. Our own negative findings recall the conclusions of Höjer (1924) that the changes in the adrenals occur 'not earlier than in other parenchymatous organs. These changes must, therefore, by no means be taken as a reason for placing the adrenals in the centre of the pathology of scurvy.'

SUMMARY

1. In two separate experiments, groups of ten or twelve guinea-pigs were maintained for 20 days on diets containing various graded intakes of ascorbic acid, ranging from 0 to 50 mg daily, or cabbage, 15 g daily. During the second half of this experimental period, one-half of the animals received, in addition to the ascorbic acid, injections, twice daily, of cortisone acetate (15 mg daily, in two doses of 7·5 mg each), whereas the remaining half had dummy injections. On the day following the final injection, the animals were killed and the adrenals analysed for ascorbic acid.

2. As was to be expected, the concentration of ascorbic acid in the adrenals was increased with each increase in the level of the ascorbic acid in the diet.

3. At all levels of vitamin C intake, the adrenal ascorbic-acid concentration was found to be lower in the cortisone-treated animals than in the corresponding dummy controls.

4. Since the effect of the cortisone was to cause a diminution in the adrenal weight, its influence in diminishing the adrenal ascorbic acid was even more pronounced when this was expressed in terms of the total amount present in the glands rather than in terms of concentration.

5. In a parallel experiment, but with a lower dose of cortisone, 7·5 mg daily, in two doses, and graded levels of ascorbic acid (0–4 mg, or as 15 g cabbage daily), a similar fall in adrenal ascorbic acid was again observed for most groups and for the experiment as a whole.

6. In a correspondingly designed experiment with ACTH (8 mg daily, in four doses, for 10 days), administered at seven graded levels of intake of vitamin C (0–50 mg, or as 15 g cabbage daily), a diminution in the concentration of ascorbic acid

in the adrenals was likewise caused, compared with the controls; but the glands were greatly enlarged by the treatment, and the total quantity present in them was raised.

7. The above findings are supported by the fact that nearly half the mean decreases (or increases) were found individually significant by statistical analysis, and that a 'combination of probabilities' test showed that the aggregate results were most unlikely to be due to chance.

8. The decrease in adrenal weight produced by cortisone, and the increase produced by ACTH, were independent of the level of intake of vitamin C, under the conditions of these tests.

We are greatly indebted to Dr J. Wishart, Cambridge University Reader in Statistics, for his kindness in supervising the statistical analyses, and to Mr D. A. East, also of the University Statistical Laboratory, who generously gave much help in the course of the calculations.

REFERENCES

Bland, M. N., Constable, B. J., Harris, L. J. & Hughes, R. E. (1952*a*). *Biochem. J.* **51**, xxxv.
Bland, M. N., Constable, B. J., Harris, L. J. & Hughes, R. E. (1952*b*). *Congr. int. Biochim.* II. *Paris. Résumés des Communications*, p. 325.
Cherry, C. P., Bland, M. N., Constable, B. J., Hughes, R. E. & Harris, L. J. (1954). *Brit. J. exp. Path.* **35**, 551.
Constable, B. J., Harris, L. J. & Hughes, R. E. (1955). *Proc. Nutr. Soc.* **14**, ix.
Fisher, R. A. (1954). *Statistical Methods for Research Workers*, 12th ed., para. 21, p. 1. Edinburgh: Oliver and Boyd.
Harris, L. J., Bland, M. N., Hughes, R. E. & Constable, B. J. (1953). *Lancet*, **264**, 1021.
Harris, L. J., Constable, B. J., Hughes, R. E. & Loewi, G. (1955*a*). *Proc. Nutr. Soc.* **14**, ix.
Harris, L. J., Constable, B. J., Hughes, R. E. & Loewi, G. (1955*b*). *Brit. J. Nutr.* **9**, 310.
Höjer, J. A. (1924). *Acta paediat., Stockh.*, **3**, suppl. p. 8.
Hughes, R. E., Harris, L. J., Constable, B. J. & Bland, M. N. (1952). *Biochem. J.* **51**, xxxv.
Ingle, D. J. & Kendall, E. C. (1937). *Science*, **86**, 245.
Ingle, D. J. & Mason, H. L. (1938). *Proc. Soc. exp. Biol., N.Y.*, **39**, 154.
Long, C. N. H. (1952). *Recent Progr. Hormone Res.* **7**, 86.
Sayers, G., Sayers, M. A., Lewis, H. L. & Long, C. N. H. (1944). *Proc. Soc. exp. Biol., N.Y.*, **55**, 238.
Sayers, M. A. & Sayers, G. (1946). *Fed. Proc.* **5**, 200.