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The Metabolism of Adrenocorticotrophic Hormone and Ascorbic Acid in the Chick

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Depletion of adrenal ascorbic acid follows the administration of adrenocorticotrophic hormone in the rat or guinea pig but not in the chick (Jailer & Boaz, 1950), quail (Zarrow & Baldini, 1952) or duckling (Zarrow & Zarrow, 1950). These authors found that continued injections of adrenocorticotrophic hormone or a 'stress agent' such as stilboestrol did nevertheless produce adrenal hypertrophy in birds. They therefore concluded that the synthesis and release of adrenocortical hormones could occur without an effect on the adrenal ascorbic acid. This paper describes a further investigation of the influence of adrenocorticotrophic hormone and stress agents on ascorbic acid metabolism in the chick.

METHODS

Animals. Cockerels, 6–10 weeks old, obtained from local breeders and kept in wire cages in a chicken shed for about a week before experiment, were fed on a commercial chick crumb diet mixed with 5% of dried-grass meal. The birds used were Light Sussex × Rhode-Island Red, Light Sussex × North-Holland Blue and North-Holland Blue strains. In each individual experiment only one strain was used.

Male Wistar rats, laboratory bred, of 200–300 g. body wt. were used.

Male guinea pigs, of approx. 250 g. body wt., were obtained from Allington Farm, Ministry of Supply, Porton, Wilts, and before being placed on experiment were fed with a fish-meal diet (20% of protein) (Harris, Constable, Howard & Leader, 1956) supplemented by 50 mg. of ascorbic acid/guinea pig/day (orally). When the guinea pigs reached a body weight of 300 g., enough casein (Glaxo Laboratories Ltd., Greenford, Middlesex) was added to the diet to increase the protein content to 55%; the diet was damped immediately before use.

Adrenocorticotrophic hormone (ACTH). Lyophilized ACTH (Armour and Co. Ltd., Chicago, Ill., U.S.A.) was injected as a solution in saline (25 i.u./2·5 ml. of 0·9% sodium chloride soln.) when a single dose was given. Since a gelatin medium is said to enhance ACTH activity when given over long periods (Wolfson, 1953) a 16% gelatin solution of the hormone [25 i.u. dissolved in 0·5 ml. of saline, followed by addition of 2 ml. of 20% (w/v) gelatin] was used when several doses were to be administered.

Analytical methods. All animals were killed swiftly by dislocation of the cervical vertebrae. This was followed by decapitation and exsanguination. Whole blood from the jugular vein was collected carefully into a receptable containing a few crystals of sodium oxalate. The organs to be analysed were rapidly excised and estimations carried out immediately. Ascorbic acid was estimated in the adrenals and plasma by the method of Mindlin & Butler (1937) and in whole blood and plasma by that of Roe & Kuether (1943); adrenal cholesterol by the ferric chloride method of Zlatkis, Zak & Boyle (1953) as described by Knobil, Hagney, Wilder & Briggs (1954); liver glycogen by the method of Good, Kramer & Somogyi (1933) as modified by Somogyi (1937) and blood glucose as described by King (1951).

RESULTS

Effect of a single injection of adrenocorticotrophic hormone on adrenal ascorbic acid

ACTH (10 i.u.) injected intramuscularly into the rat produced, 1 hr. after injection, a 30 % decrease in adrenal ascorbic acid concentration as compared with control animals injected with saline. In the chick, however, results were obtained similar to those of Jailer & Boaz (1950). Both intramuscular and intravenous injections had no effect 1, 2 or 6 hr. after injection (Table 1). After 6 hr., however, a 20 % depletion of adrenal cholesterol occurred, a phenomenon which in the rat and guinea pig accompanies the fall in ascorbic acid produced by ACTH (Long, 1946).

Effect of adrenocorticotrophic hormone on carbohydrate metabolism

The deposition of liver glycogen which occurs in the adrenalectomized fasted rat or mouse after administration of adrenal hormones, is the basis of a method for their bioassay (Reinecke & Kendall, 1942; Venning, Kazmin & Bell, 1946). Blood-sugar levels are also elevated by the influence of these hormones. Since a primary effect of ACTH is to stimulate production of adrenocorticoids, it was of interest to determine whether these glycogenic properties could be induced by injections of ACTH in the fasted intact animal.

Cockerels were fasted for 24 hr. but allowed access to water. One group was then injected with five doses of ACTH (5 i.u. in 16 % gelatin), at 1 hr. intervals, and killed 1 hr. after the last injection. A control group was injected likewise with 16 % gelatin. On analysis, chicks receiving ACTH were found to have 20 times more liver glycogen and 25 % more blood glucose than control animals (Table 2), although their adrenal ascorbic acid concentration was unchanged.

According to Olsen *et al.* (1944) the glycogenic response to adrenocorticoids in rats is increased by feeding a diet high in protein. In our preliminary experiments with guinea pigs the response to ACTH was also found to be enhanced by pre-feeding a high-protein diet. Guinea pigs were therefore fed with a 55 % protein diet for 7 days before experiment, deprived of food for 24 hr. and then injected with five 1-hourly doses of ACTH (2 i.u. in 16 % gelatin) as described above. Liver glycogen and blood glucose were found to be increased compared with controls injected with gelatin solution without ACTH (Table 2). In contrast with chicks, however, there was a concurrent fall in adrenal ascorbic acid concentration.

Table 1. *Effect of a single injection of adrenocorticotrophic hormone* (10 *i.u.*) *on adrenal ascorbic acid and cholesterol in the chick compared with the rat*

In this and in the other tables all results are given ± S.E. Values in which the difference from normal is statistically significant are denoted by asterisks.

Animal	Injection	Mode of injection	Time killed after injection (hr.)	No. of animals	Adrenal ascorbic acid (mg./100 g.)	Adrenal cholesterol (g./100 g.)	Blood ascorbic acid (mg./100 ml.)
Rat	Saline	Intramuscular	1	6	347 ± 23	—	—
	ACTH	Intramuscular	1	6	246 ± 20*	—	—
Chick	Saline	Intravenous	1	4	144 ± 5·9	—	—
	ACTH	Intravenous	1	4	136 ± 14·9	—	—
	Saline	Intravenous	6	4	138 ± 6·5	5·38 ± 0·13	1·16 ± 0·17
	ACTH	Intravenous	6	4	155 ± 4·3	3·85 ± 0·29**	1·18 ± 0·15
	Saline	Intramuscular	2	8	148 ± 5·1	—	—
	ACTH	Intramuscular	2	8	145 ± 6·8	—	—

* Denotes $P = 0{\cdot}05$–$0{\cdot}01$. ** Denotes $P = 0{\cdot}01$–$0{\cdot}001$.

Table 2. *Effects of five* 1*-hourly injections of adrenocorticotrophic hormone in fasted chicks and guinea pigs*

Each chick received a total of 25 i.u., and each guinea pig a total of 10 i.u. of ACTH.

Metabolite	Treatment	Chick†	Guinea pig‡
Liver glycogen (mg./100 g.)	Control	9 ± 4·1	102 ± 22
	ACTH	204 ± 45**	407 ± 114*
Blood glucose (mg./100 ml.)	Control	165 ± 10·8	99 ± 2·1
	ACTH	209 ± 6·0**	116 ± 3·5**
Adrenal ascorbic acid (mg./100 g.)	Control	176 ± 10·5	89·1 ± 3·9
	ACTH	172 ± 7·1	56 ± 6·6**

* Denotes $P = 0{\cdot}05$–$0{\cdot}01$. ** Denotes $P = 0{\cdot}01$–$0{\cdot}001$. † 8 animals/group. ‡ 6 animals/group.

Table 3. *Effects in chicks of continuous injections of adrenocorticotrophic hormone*

Chicks received 15 i.u. of ACTH/day for 8 days.

Treatment	No. of animals	Body wt. At start (g.)	Body wt. At end (g.)	Liver wt. (g.)	Adrenal wt. (mg.)	Adrenal cholesterol (g./100 g.)	Adrenal ascorbic acid (mg./100 g.)	Blood ascorbic acid (mg./100 ml.)
Control	6	891±22	1103±16	33·7±3·0	98±5·0	3·64±0·19	121±5·1	1·46±0·10
ACTH	6	887±19	951±18*	30·5±1·3	134±10·7**	1·69±0·10***	115±9·3	0·70±0·06***

* Denotes $P = 0{\cdot}05$–$0{\cdot}01$. ** Denotes $P = 0{\cdot}01$–$0{\cdot}001$. *** Denotes $P = <0{\cdot}001$.

Table 4. *Effect of pituitary stimulants on adrenal ascorbic acid in the rat and chick [with significance (P) between non-injected and injected animals]*

Animal	Injection	No. of animals	Adrenal ascorbic acid (mg./100 g.)
Rat	None	6	444±15
	Saline	6	348±23**
	Histamine	6	264± 7***
	Insulin	6	202±18***
Chick	None	7	115± 3
	Saline	6	121± 9
	Histamine	5	110± 3
	Insulin	6	111± 8

* Denotes $P = 0{\cdot}05$–$0{\cdot}01$.
** Denotes $P = 0{\cdot}01$–$0{\cdot}001$.
*** Denotes $P = <0{\cdot}001$.

Continued administration of adrenocorticotrophic hormone

Administration of ACTH to guinea pigs over a long period has been shown previously to result in enlargement of the adrenal gland, and a diminution in adrenal ascorbic acid concentration (Constable, Harris & Hughes, 1955). An increased liver weight also occurs in the guinea pig and rabbit, although not in the rat, chick or mouse (Harris, Bland, Hughes & Constable, 1953). In a continuation of these studies, cockerels were injected with ACTH (15 i.u. in 16 % gelatin) in divided doses twice daily for 8 days. A second group was similarly treated with gelatin solution without ACTH. As shown in Table 3, ACTH treatment caused a retardation in growth, an enlargement of the adrenals and a greatly diminished adrenal cholesterol concentration. Although there was no change in adrenal ascorbic acid concentration, an unexpected result was a 50 % diminution in the ascorbic acid content of the blood. A single injection of ACTH had no observable effect on blood ascorbic acid (Table 1). As noted previously, ACTH did not produce enlargement of the liver in the chick (Harris *et al.* 1953).

Pituitary stimulants and adrenal ascorbic acid

Injections of numerous substances are known to produce a fall in adrenal ascorbic acid in the intact rat. Since this effect is absent in the hypophysectomized animal, they are thought to act through the stimulation of the hypophysis and production of endogenous ACTH (Sayers & Sayers, 1948). In our experiments, groups of six rats were injected with saline at room temperature (0·05 ml./100 g. body wt.), histamine diphosphate (1·5 mg./100 g. in 0·05 ml. of saline) and insulin (2·5 i.u./100 g. in 0·05 ml.). In all instances the injected animals had a lower adrenal ascorbic acid concentration than non-injected controls (Table 4). The effect of histamine and of insulin was also greater than that of saline alone. No evidence was obtained that the above-mentioned phenomenon occurs in chicks, since injections of similar doses of these substances had no effect on their adrenal ascorbic acid (Table 4).

Sodium salicylate and ascorbic acid

The administration of salicylate also depletes the adrenal ascorbic acid of the rat, but it is thought that in this instance endogenous ACTH is not entirely responsible, since the effect occurs even in the hypophysectomized animal (Coste, Bourrel & Delbarre, 1953; Weidmann, 1955). In our experiments on the rat, an intramuscular injection of sodium salicylate (20 mg./100 g. in 0·1 ml. of saline) produced a marked decrease in adrenal ascorbic acid 2 hr. later (Table 5). With the same dose, however, no effect was observed in the chick. When a much larger dose (60 mg./100 g.) was administered to chicks, a small but significant decrease did occur. As shown in Table 5, with this dose there was also a rise in plasma ascorbic acid, as determined by two different methods.

DISCUSSION

The sharp fall of ascorbic acid in the rat or guinea-pig adrenal after the administration of ACTH (Sayers, Sayers, Lewis & Long, 1944) has been utilized for its assay. Although there is ample evidence that this effect is associated with an increased output of adrenal steroids (Bush, 1953), it appears to be confined to mammalian species. We have confirmed the observation of Jailer & Boaz (1950) that a single injection of ACTH is

Table 5. *Effect of injections of sodium salicylate on adrenal and plasma ascorbic acid in rats and chicks*

Plasma ascorbic acid was measured as described by Mindlin & Butler (1937) (*A*) and by Roe & Kuether (1943) (*B*).

			Ascorbic acid		
				Plasma	
Animal	Injection	No. of animals	Adrenals (mg./100 g.)	(*A*) (mg./100 ml.)	(*B*) (mg./100 ml.)
Rat	Saline	6	348±10·1	—	—
	Salicylate (20 mg./100 g. body wt.)	6	228±10·7***	—	—
Chick	None	7	115± 3·0	—	—
	Salicylate (20 mg./100 g.)	6	111± 4·0	—	—
	Saline	9	121± 9·3	1·56±0·08	1·55±0·10
	Salicylate (60 mg./100 g.)	9	90± 6·3*	2·19±0·07***	2·06±0·07***

* Denotes $P = 0{\cdot}05$–$0{\cdot}01$. ** Denotes $P = 0{\cdot}01$–$0{\cdot}001$. *** Denotes $P = <0{\cdot}001$.

without effect on the adrenal ascorbic acid of chicks. We find that the depletion of adrenal cholesterol, which is a characteristic result of administration of ACTH to mammals, also occurs in the chick. Since adrenal cholesterol is considered to be a precursor of the adrenal corticoids, this finding suggests that they can in fact be synthesized in birds under the stimulation of the pituitary hormone. Further evidence was obtained by studying the effects of ACTH on carbohydrate metabolism. The administration of cortical hormones is known to produce an increase in liver glycogen and elevated blood-sugar levels in fasted chicks, as in other species (Golden & Long, 1942). Similar effects were observed in guinea pigs and chicks with ACTH. But whereas in guinea pigs a significant fall in adrenal ascorbic acid concentration was produced, this effect was absent in the chick. It can thus be inferred that ACTH is capable of stimulating the production of adrenal hormones in chicks without affecting adrenal ascorbic acid. It is unwise to conclude from these results that ascorbic acid is unnecessary for the synthesis of the adrenocorticoids, but the evidence is strongly suggestive. The elevated levels of adrenocorticoids found in the plasma (Done, Ely, Heiselt & Kelly, 1953) and in the urine (Burstein, Dorfman & Nadel, 1955) of scorbutic guinea pigs provide evidence to support this view.

As in other species, continued administration of ACTH to chicks resulted in an enlargement of the adrenals accompanied by a very low cholesterol content. This latter finding is usually associated with exhaustive stimulation of the adrenal (Sayers & Sayers, 1948). As in the rat (Asling, Reinhardt & Li, 1950), there is a retardation in growth. A surprising result was the greatly diminished concentration of ascorbic acid in the blood of chicks produced by prolonged injection of ACTH, an effect which was not apparent after a single injection. Further work is necessary before it can be established whether or not this effect is due to a direct interaction of ACTH and ascorbic acid. The concentration of ascorbic acid in the adrenals was unaffected.

Substances known to produce endogenous ACTH in the rat, such as histamine, insulin and saline, were without effect on adrenal ascorbic acid in the chick. In this respect our results agree with those of earlier workers who used adrenaline (Jailer & Boaz, 1950) and malarial infection (Taylor, 1952) as pituitary stimulants.

According to Cronheim, King & Hyder (1952) sodium salicylate (30 mg./100 g. body weight) acts as a pituitary stimulant in rats, and the ascorbic acid-depleting effect is prevented by hypophysectomy. On the other hand Coste *et al.* (1953), using a higher dose (50 mg./100 g.), found that salicylate is capable of eliciting a response even when the rat is hypophysectomized. Weidmann (1955) found that the depletion of adrenal ascorbic acid in hypophysectomized rats varied with the dose given. Coste *et al.* and Weidmann suggest that salicylate has a direct action on adrenal ascorbic acid. Our results agree with this view since we have found that in chicks a high dose of salicylate (60 mg./100 g.) was capable of depleting ascorbic acid, although a lower dose (30 mg./100 g.), which was active in normal rats, was ineffective. Moreover, the changes in ascorbic acid metabolism induced by high levels of salicylate were not confined to the adrenal since elevated plasma ascorbic acid was also observed. A rise in plasma ascorbic acid has also been noted in man dosed orally with salicylate (Stewart, Horn & Redson, 1953), and although the mechanism seems obscure the result is interesting in view of the chemical interaction *in vitro* of salicylates with ascorbic acid, involving hydroxylation of the aromatic nucleus (Brodie, Axelrod, Shore & Udenfriend, 1954).

SUMMARY

1. It has been confirmed that the transient fall in adrenal ascorbic acid after the administration of adrenocorticotrophic hormone (ACTH) in the rat does not occur in the chick.

2. A single injection of ACTH resulted in a depletion of adrenal cholesterol in chicks.

3. Evidence has been obtained that in the chick ACTH can stimulate the production of adrenal hormones affecting carbohydrate metabolism although having no effect on adrenal ascorbic acid.

4. The continued administration of ACTH to the chick also caused a retardation of growth, an enlargement of the adrenal and a decreased adrenal cholesterol concentration. Although the ascorbic acid was unaffected in the adrenal there was a fall in its concentration in the blood.

5. No evidence was obtained that endogenous ACTH produced by pituitary stimulants could deplete adrenal ascorbic acid in the chick.

6. Large doses of sodium salicylate caused the ascorbic acid to fall in rat and chicken adrenals and to rise in chicken plasma.

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Studies in the Biochemistry of Micro-organisms

105. CHEMICAL DEGRADATION OF SCLEROTIORIN*

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Sclerotiorin, a metabolic product originally obtained from the mycelium of *Penicillium sclerotiorum* by Curtin & Reilly (1940), was isolated by us (Birkinshaw, 1952) in considerably larger yield from *Penicillium multicolor*. The molecular formula put forward by Curtin & Reilly was corrected to $C_{21}H_{23}O_5Cl$ and the unsaturated acid obtained on alkaline hydrolysis was identified as (+)-4:6-dimethylocta-2:4-dienoic acid. This structure for the unsaturated acid was also arrived at independently by Watanabe (1952).

The work was continued and some products resulting from further degradation of sclerotiorin, and particularly of its compound with ammonia, indicating a possible partial structure, were mentioned in a communication (Birkinshaw, 1956) in

* Part 104: Raistrick & Stössl (1958).