Specificity of Action of Adrenocorticotrophin *in vitro* on Ascorbate Transport in Rat Adrenal Glands

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The inhibition of ascorbate transport by rat adrenal quarters in response to steroidogenesis *in vitro* was shown to be highly specific with respect to tissue, substrate and steroidogenic agent. The transport system *in vitro* is capable of net accumulation of ascorbate. The evidence is consistent with the conclusion that ascorbate 'depletion' in the adrenal gland is due to a specific block by corticoids of the uptake of ascorbate.

The use of the ascorbate-'depletion' test for many years as an assay for ACTH* suggests that there is a highly specific relationship between ACTH and adrenal ascorbate. Since the major metabolic effect of ACTH is to stimulate the production of corticoids, it would not be surprising if there were a functional relationship between corticoids and the content of ascorbate in the adrenal gland. The relationship between steroidogenesis and ascorbate 'depletion', however, remains obscure.

It has been shown that in the adrenal glands of rat and guinea pig there is an energy-dependent uptake of ascorbate that is inhibited by ACTH and corticoids *in vitro* (Sharma, Johnstone & Quastel, 1963; Sharma, Johnstone & Quastel, 1964; de Nicola, Clayman & Johnstone, 1968a). The content of ascorbate found in the adrenal gland of different species can be correlated with the ability of that tissue to transport [$1\text{-}^{14}\text{C}$]ascorbate *in vitro* and it is proposed that the high tissue content of ascorbate in the gland is the result of an active transport system (de Nicola, Clayman & Johnstone, 1968b).

It is proposed further that the 'depletion' phenomenon observed *in vitro* results from an inhibition of the transport of ascorbate by corticoids produced in response to ACTH (Sharma *et al.* 1963, 1964; de Nicola *et al.* 1968a). The ascorbate-'depletion' response *in vivo* is highly specific (Sayers, Sayers, Liang & Long, 1946; Sayers, Sayers & Woodbury, 1948). It occurs in the adrenal gland of hypophysectomized animals only in response to ACTH and it is not known to occur in other ascorbate-containing organs in response to ACTH. The object of this study was to show that inhibition of ascorbate transport *in vitro* occurs in a highly specific manner and that the specificity observed *in vitro* is similar in nature to the 'depletion'

phenomenon *in vivo* in a manner consistent with the conclusion that the same process is being measured.

MATERIALS AND METHODS

The preparation and handling of the rat adrenal and pituitary glands have been described (de Nicola *et al.* 1968a; Clayman, Tsang & Johnstone, 1970). The incubation medium (Krebs-Ringer phosphate) consisted of 157 mM- Na^+ , 5.8 mM- K^+ , 152.0 mM- Cl^- , 3 mM- Ca^{2+} , 1.7 mM- MgSO_4 , 10 mM-glucose and 10 mM-sodium phosphate buffer, pH 7.4. Incubation was carried out at 37°C in O_2 . A preincubation period of 45 min at 37°C was used as described by Saffran & Schally (1955).

In all uptake studies, extracellular spaces were measured as described by Kipnis & Parrish (1965) and corrections made for extracellular water. Amino acid uptake was measured as described for the uptake of [$1\text{-}^{14}\text{C}$]ascorbate (de Nicola *et al.* 1968a). The procedure requires the extraction of the tissue in 85% (v/v) ethanol or 5% (w/v) trichloroacetic acid followed by centrifugation and measurement of the radioactivity of a sample of the clear extract. Incorporation of amino acids into proteins was measured by a modification of the Schneider procedure (Schneider, 1945; Coles, Johnstone & Quastel, 1960). Steroid production was measured in a methylene dichloride extract of the medium by the extinction at 240 nm or by the tetrazolium method (Saffran & Schally, 1955; Elliott, Birmingham, Schally & Schonbaum, 1954). Ascorbate analyses were performed with a Technicon AutoAnalyzer (Nesset, Windsor, Humphrey & Callantine, 1967). The adrenal glands were extracted in 2.5% (w/v) metaphosphoric acid and the analyses for ascorbate were performed within 1 h after extraction of the tissue.

In studies with amino acids, the identity of the amino acids that accumulated in the tissue was established. The extracts were subjected to paper chromatography with butan-1-ol-acetic acid-water (5:4:1, by vol.) followed by exposure to Kodak X-Ray film. Only with serine was a component other than the original amino acid found and

* Abbreviation: ACTH, adrenocorticotrophin.

this was identified as glycine by co-chromatography with glycine in a two-dimensional chromatogram by using butan-2-ol-90% formic acid-water (20:3:5, by vol.) in the first dimension and 2-methylpropan-2-ol-butan-2-one-aq. ammonia (sp.gr. 0.88)-water (10:10:3:5, by vol.) in the second dimension. The radioactive areas were located by radioautography, eluted and the radioactivity was measured. After incubation with [^{14}C]₃serine for 2 h at 37°C, 20–30% of the radioactivity in the gland was found in glycine.

Most radioactive measurements were performed with a Packard Scintillation counter and by using Bray's (1960) solvent. Radioactivity in proteins, however, was measured by a planchet method (Coles *et al.* 1960) with a Nuclear-Chicago thin-end-window flow counter (efficiency 30%).

Materials. The peptide hormones, except ACTH, were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. ACTH was kindly supplied by the National Institute for Medical Research, London N.W.7, U.K. Compounds

labelled with ^{14}C were purchased from New England Nuclear Corp., Boston, Mass., U.S.A.

RESULTS AND DISCUSSION

It has been shown that the transport of many organic compounds against a concentration gradient shows very similar requirements for metabolic energy (or ATP), 100–150 mM- Na^+ and 5 mM- K^+ in the external medium (Riklis & Quastel, 1958; Crane, 1965; Johnstone & Scholefield, 1965; Albers, 1967). In several cases the ionic requirements have been associated with the necessity for ion transport (Schultz & Zalusky, 1963; Schultz, Fuisz & Curran, 1966). It is therefore evident that any change in metabolic conditions that would alter the parameters cited would affect active transport systems in general. If the effects of ACTH or corticoids

Table 1. *Effect of metabolic conditions on amino acid uptake in quartered rat adrenal gland*

The adrenal glands were incubated for 2 h in the incubation medium containing Krebs-Ringer phosphate (KRP) and 10 mM-glucose as specified in the Materials and Methods section, under a gas phase of pure O_2 or N_2 . The incubation period followed a 45 min preincubation. For taurine the incubation period was 3 h. The incubation volume was 1.0 ml. In sucrose media, iso-osmotic sucrose replaced the NaCl. The buffer remained 10 mM-sodium phosphate, pH 7.4, giving a final concentration of 18 mM- Na^+ . NaF was used at a concentration of 10 mM. The results are given as means $\pm$ s.e.m. with the numbers of experimental values in parentheses. The concentration ratios were calculated by the method of Kipnis & Parrish (1965), as described by de Nicola *et al.* (1968a). Specific radioactivities of amino acids used were: [^{14}C]glycine, 5.5×10^6 c.p.m./ μmol ; L-[^{14}C]₃serine, 1.2×10^8 c.p.m./ μmol ; L-[^{14}C]₄threonine, 1.8×10^8 c.p.m./ μmol ; [1,2- ^{14}C]taurine, 10^6 c.p.m./ μmol ; L-[^{14}C]₆lysine, 1.2×10^5 c.p.m./ μmol .

Amino acid	Gas phase	Medium	Amino acid uptake (c.p.m./ml of cell water) (c.p.m./ml of medium)
Glycine (10 μM)	O_2	KRP (normal)	4.4 ± 0.3 (5)
	N_2	KRP	2.3 ± 0.1 (5)
	N_2	KRP (+ NaF)	1.6 ± 0.1 (5)
	O_2	Sucrose medium	2.2 ± 0.1 (8)
		(Na^+ -free)	
Taurine (0.1 mM)	O_2	KRP (K^+ -free)	3.1 ± 0.3 (4)
	O_2	KRP	13.7 ± 0.15 (5)
	N_2	KRP	6.3 ± 0.10 (5)
	N_2	KRP (+ NaF)	3.0 ± 0.4 (5)
	O_2	Sucrose medium	2.8 ± 0.1 (5)
L-Serine (1 μM)		(Na^+ -free)	
	O_2	KRP (K^+ -free)	7.3 ± 2.0 (4)
	O_2	KRP	5.6 ± 0.1 (5)
	N_2	KRP	5.4 ± 0.25 (5)
	N_2	KRP (+ NaF)	2.2 ± 0.3 (4)
L-Lysine (1 mM)	O_2	Sucrose medium	2.9 ± 0.2 (4)
		(Na^+ -free)	
	O_2	KRP (K^+ -free)	3.5 ± 0.4 (4)
	O_2	KRP	3.4 ± 0.2 (5)
	N_2	KRP	3.4 ± 0.3 (5)
	N_2	KRP (+ NaF)	1.6 ± 0.2 (5)
	O_2	Sucrose medium	2.4 ± 0.2 (5)
		(Na^+ -free)	
	O_2	KRP (K^+ -free)	2.8 ± 0.2 (5)

depend on alterations in energy supply, ionic balance, or both, the transport of other organic compounds should be affected. However, if the effect of the hormones is directed specifically against ascorbate, the inhibition of transport should be restricted to ascorbate.

For these comparative studies we examined a number of amino acids and carbohydrates. As a rule we did not find accumulation of carbohydrate derivatives against a concentration gradient in the adrenal gland, but a number of amino acids, particularly taurine, were extremely well accumulated and subject to the same general metabolic influences as ascorbate transport. Taurine had the additional advantage of not being incorporated into proteins so that the interpretation of the results was less complicated.

Initially all amino acids were examined at several concentrations between $1\mu\text{M}$ and 1mM . With each amino acid, the concentration finally selected for critical experiments was the highest concentration with which high intracellular levels of radioactivity (minimum 500 c.p.m./sample) were found coupled with a high concentration ratio between cell water and medium. For example, with serine, as the concentration was increased to 1mM from $1\mu\text{M}$ the radioactivity in the tissue increased but the concentration ratio decreased from 5.0 to 1.5. Since we could obtain at least 500 c.p.m./sample with $1\mu\text{M}$ -serine and the concentration ratio obtained was between 5 and 6, we chose this concentration

rather than 1mM . With lysine, the concentration ratio did not increase so markedly when concentrations below 1mM were used. No doubt these differences reflect differences in the extent to which an amino acid is accumulated as well as its K_m value for transport (the concentration at which half-maximal rate of transport is obtained).

Table 1 shows that anaerobiosis, as well as decreased extracellular concentrations of Na^+ and K^+ ions, result in a substantial inhibition of amino acid accumulation. These results are similar to those reported for $[1-^{14}\text{C}]$ ascorbate uptake (Sharma *et al.* 1964; de Nicola *et al.* 1968a) and serine uptake in guinea-pig adrenal gland (Sharma *et al.* 1964). With some amino acids (e.g. lysine, serine and threonine) uptake under anaerobic conditions is not different from that observed aerobically unless sodium fluoride is added. Presumably sufficient energy can be obtained from glycolytic mechanisms to supply these transport systems. Thus any conditions that affect transport by interfering with fundamental processes such as energy production and ion distributions would not discriminate between ascorbate transport and energy-dependent, ion-dependent amino acid transport. It may be noted that Salomon (1947) observed ascorbate 'depletion' in the adrenal gland of hypophysectomized rats during transient anoxia.

Table 2 shows that neither corticoids at 0.1mM nor ACTH at 100m-units/ml nor 10mM $3':5'$ -cyclic AMP interfere with the uptake of several amino

Table 2. Action of ACTH, $3':5'$ -cyclic AMP and steroids on amino acid uptake in the rat adrenal gland in vitro

Conditions of incubation were as described for Table 1. The gas phase was pure O_2 in Krebs-Ringer phosphate medium. Tissue from the same group of animals was used with and without the 'addition' in every case, but not necessarily with every transported substance. Steroids were measured in the incubation medium (Elliott *et al.* 1954). The results are given as means $\pm$ s.e.m. with the numbers of experimental values in parentheses. The values for ascorbate uptake under comparable conditions were: ACTH (100m-units/ml): control, 7.8 ± 0.3 (5); plus addition, 5.1 ± 3 (5); $3':5'$ -cyclic AMP (10mM): control, 8.2 ± 0.9 (4); plus addition, 4.8 ± 0.6 (4); corticosterone (0.1mM): control, 7.6 ± 0.1 (5); plus addition, 5.5 ± 0.2 (5). These values are taken from the data of de Nicola *et al.* (1968a).

Amino acid	Addition	Uptake of amino acid (c.p.m./ml of cell water) (c.p.m./ml of medium)		Steroid production ($\mu\text{g}/100\text{mg}$ of tissue)	
		No addition	Plus addition	No addition	Plus addition
Glycine	ACTH (100m-units)	5.5 ± 0.1 (4)	5.3 ± 0.1 (4)	14.0 ± 0.2 (4)	31.0 ± 0.6 (9)
	ACTH (500m-units)	5.5 ± 0.1 (5)	5.8 ± 0.2 (5)	14.0 ± 0.2 (5)	43.0 ± 1.9 (5)
Taurine	ACTH (100m-units)	12.7 ± 0.6 (5)	13.1 ± 0.4 (5)	12.7 ± 0.6 (5)	28.1 ± 0.4 (5)
	ACTH (500m-units)	5.7 ± 0.1 (4)	5.7 ± 0.1 (4)	9.8 ± 1.2 (4)	35.8 ± 1.7 (4)
Glycine	$3':5'$ -Cyclic AMP (10mM)	4.8 ± 0.2 (5)	4.9 ± 0.4 (5)	10.7 ± 1.2 (5)	44.0 ± 2.3 (5)
Taurine	$3':5'$ -Cyclic AMP (10mM)	13.2 ± 0.4 (3)	12.9 ± 0.7 (5)	10.7 ± 1.1 (3)	42.5 ± 2.3 (5)
Serine	$3':5'$ -Cyclic AMP (10mM)	5.9 ± 0.1 (3)	6.3 ± 0.3 (3)	8.2 ± 1.2 (3)	33.4 ± 2.3 (3)
Glycine	Corticosterone (0.1mM)	4.6 ± 0.3 (5)	4.9 ± 0.1 (5)		
Serine	Corticosterone (0.1mM)	5.7 ± 0.3 (4)	6.1 ± 0.3 (4)		
Glycine	Aldosterone (0.1mM)	4.7 ± 0.3 (5)	4.9 ± 0.1 (5)		
Serine	Aldosterone (0.1mM)	6.2 ± 0.2 (5)	5.9 ± 0.2 (5)		

Table 3. *Inhibition of ascorbate transport by pituitary hormones*

The conditions of incubation were as described for Table 2. L-[1-¹⁴C]Ascorbic acid (0.1 mM; specific radioactivity 10⁶ c.p.m./μmol) in 1 mM-thiourea was present throughout. Incubation was for 2.5 h at 37°C in the normal incubation medium. The steroids were assayed in the incubation medium. The results are given as means ± s.e.m. with the numbers of experimental values in parentheses. Hormone concentrations: ACTH, 1 μg/ml; follicle-stimulating hormone (FSH), 20 μg/ml; luteinizing hormone (LH), 20 μg/ml; growth hormone (GH), 20 μg/ml.

Conditions	Ascorbate uptake	Steroid production ($\mu\text{g}/100\text{ mg}$ of tissue)
	$\left(\frac{\text{c.p.m./ml of cell water}}{\text{c.p.m./ml of incubation medium}}\right)$	
Intact rats		
No ACTH	5.9 ± 0.1 (6)	10.6 ± 0.6 (6)
+ ACTH	3.7 ± 0.1 (6)	24.4 ± 0.8 (6)
+ LH	5.9 ± 0.3 (3)	No change from control
+ GH	5.6 ± 0.2 (3)	No change from control
+ FSH	5.3 ± 0.3 (3)	No change from control
20 h after hypophysectomy		
No ACTH	5.6 ± 0.2 (6)	8.0 ± 0.1 (6)
+ ACTH	4.1 ± 0.1 (6)	18.8 ± 0.2 (6)
48 h after hypophysectomy		
No ACTH	3.1 ± 0.2 (6)	7.5 ± 0.1 (6)
+ ACTH	3.3 ± 0.2 (6)	9.5 ± 1.0 (6)

acids. This is in contrast with the earlier results on ascorbate uptake which are included for comparative purposes (de Nicola *et al.* 1968a). Clearly then there is no generalized inhibition of transport in the presence of ACTH, 3':5'-cyclic AMP or corticoids at these concentrations.

We also examined the uptake of a number of sugars or sugar-like compounds such as 3-O-methyl-D-glucose, 6-deoxy-D-glucose and myoinositol. Neither 3-O-methyl-D-glucose nor 6-deoxy-D-glucose was accumulated by the gland whereas myoinositol was accumulated. Inositol uptake, however, was not affected by ACTH. Hence inhibition of transport by ACTH, 3':5'-cyclic AMP and corticoids in the adrenal gland does not apply to all transportable substrates and appears to be restricted to ascorbate. Moreover, experiments have shown that not one of ten amino acids or five carbohydrate-like substances tested (including ascorbate analogues such as D-iso-ascorbate and dehydroascorbate) inhibits [1-¹⁴C]ascorbate uptake even when used at 10–100 times the concentration of ascorbate.

Action of pituitary peptide hormones. If the phenomenon of inhibition of ascorbate transport observed *in vitro* is analogous to the 'depletion' phenomenon *in vivo*, then the only peptide hormone to exert the inhibition should be ACTH. Table 3 shows that luteinizing hormone, growth hormone and follicle-stimulating hormones do not affect the uptake of ascorbate, whereas ACTH does. As the adrenal glands of hypophysectomized rats atrophy and cease to respond to ACTH with increased

corticoid production, inhibition of ascorbate transport by ACTH also disappears.

Amino acid uptakes *in vitro* in rat adrenal glands from hypophysectomized rats (20 h after hypophysectomy) are near the normal values. Concentration ratios for glycine and taurine are 4.5 ± 0.4 (3) and 14.2 ± 1.9 (4) respectively under optimum conditions of incubation as described in Table 2.

Action of nucleotides. It has been shown that 3':5'-cyclic AMP is produced in response to ACTH in the adrenal gland (Graham-Smith, Butcher, Ney & Sutherland, 1967) and that this nucleotide is probably the tissue mediator of the steroidogenic response (Hayes, Koritz & Peron, 1959; Birmingham, Kurlents, Lane, Muhlstock & Traikov, 1960). Table 4 shows that several nucleotides, other than cyclic derivatives, at equivalent concentration, are without effect on increasing steroid production or decreasing ascorbate uptake. Thus 3':5'-cyclic AMP is effective in this system although it (like other nucleotides) is without effect on amino acid transport (Table 4; see also Table 2). Only the cyclic nucleotide inhibits ascorbate transport and increases steroidogenesis.

Protein synthesis and steroidogenesis. It seems likely that the corticoids themselves are the specific inhibitors of ascorbate transport in the adrenal gland. Evidence has been presented for this conclusion by Sharma *et al.* (1963, 1964) and de Nicola *et al.* (1968a). Supporting evidence is that the inhibition of ascorbate transport, like stimulation of steroid production in response to 3':5'-cyclic

AMP (or ACTH; de Nicola *et al.* 1968a), requires protein synthesis. If protein synthesis is blocked by cycloheximide or puromycin neither steroid production (Ferguson, 1963; Graham-Smith *et al.* 1967; Davis & Garren, 1968) nor inhibition of ascorbate transport (de Nicola *et al.* 1968a) occurs in response to ACTH or 3':5'-cyclic AMP. However, the inhibition of ascorbate transport by the corti-

coids does not require a system capable of incorporating amino acids into proteins. Table 5 shows that in the presence of puromycin, which abolishes amino acid incorporation into proteins, the steroids maintain their ability to block the transport of [1-¹⁴C]ascorbate. It should be noted that ascorbate transport is not decreased under conditions where protein synthesis is completely blocked (Table 5

Table 4. *Action of nucleotides on the uptake of L-[1-¹⁴C]ascorbate and [1-¹⁴C]glycine in the rat adrenal gland*

Conditions of incubation were as described for Table 2. The nucleotides were all present at a concentration of 10 mM. Glycine was present at 10 μ M as described for Table 1. The results are given as means $\pm$ s.e.m., with the numbers of experimental values in parentheses.

Addition	Ascorbate uptake ($\frac{\text{c.p.m./ml of cell water}}{\text{c.p.m./ml of medium}}$)		Steroid production ($\mu\text{g}/100\text{ mg of tissue}$)	
	No addition	Plus addition	No addition	Plus addition
3'-AMP	10.7 $\pm$ 0.2 (4)	10.9 $\pm$ 0.1 (4)	9.0 $\pm$ 1.3 (4)	13.0 $\pm$ 2.1 (4)
5'-UMP	10.7 $\pm$ 0.3 (4)	10.7 $\pm$ 0.3 (4)	9.0 $\pm$ 1.3 (4)	10.2 $\pm$ 0.4 (4)
5'-GMP	10.7 $\pm$ 0.4 (4)	11.0 $\pm$ 0.4 (4)	10.2 $\pm$ 1.8 (4)	12.8 $\pm$ 1.0 (4)
5'-CMP	10.7 $\pm$ 1.2 (4)	10.6 $\pm$ 0.6 (4)	10.2 $\pm$ 1.3 (4)	12.0 $\pm$ 1.2 (4)
5'-AMP	8.6 $\pm$ 0.1 (4)	8.7 $\pm$ 0.1 (6)	9.2 $\pm$ 0.1 (4)	9.3 $\pm$ 0.9 (6)
3':5'-Cyclic AMP	9.8 $\pm$ 0.2 (6)	5.4 $\pm$ 0.2 (6)	9.0 $\pm$ 1.2 (6)	40.8 $\pm$ 1.0 (6)
Glycine uptake ($\frac{\text{c.p.m./ml of cell water}}{\text{c.p.m./ml of medium}}$)				
3'-AMP	5.2 $\pm$ 0.1 (4)	4.9 $\pm$ 0.3 (4)	9.7 $\pm$ 0.3 (4)	9.6 $\pm$ 1.2 (4)
5'-AMP	4.7 $\pm$ 0.3 (4)	4.7 $\pm$ 0.1 (4)	9.9 $\pm$ 1.0 (4)	10.7 $\pm$ 3.6 (4)

Table 5. *Protein synthesis and ascorbate transport in the rat adrenal gland*

Conditions were as described for Table 2. Pilot experiments were done separately to confirm that 1.0 mM-puromycin did block amino acid incorporation into proteins. The results are given as means $\pm$ s.e.m., with the numbers of experimental values in parentheses. In all cases greater than 90% inhibition was obtained with 1 mM-puromycin whereas only 50 $\pm$ 10% inhibition was obtained with 0.1 mM-puromycin.

Additions			Ascorbate uptake ($\frac{\text{c.p.m./ml of cell water}}{\text{c.p.m./ml of medium}}$)	Steroid production ($\mu\text{g}/100\text{ mg of tissue}$)	Inhibition of amino acid incorporation (%)
3':5'-Cyclic AMP (10 mM)	Puromycin (1 mM)	Aldosterone (0.1 mM)			
—	—	—	7.2 $\pm$ 0.3 (6)	9.5 $\pm$ 0.2 (6)	>90
—	+	—	7.2 $\pm$ 0.4 (6)	9.6 $\pm$ 0.2 (6)	
—	—	—	8.0 $\pm$ 0.4 (6)	10.2 $\pm$ 0.1 (6)	
+	—	—	4.6 $\pm$ 0.1 (6)	37.5 $\pm$ 1.5 (6)	>90
—	—	—	7.3 $\pm$ 0.4 (6)	9.6 $\pm$ 0.1 (6)	
+	+	—	6.8 $\pm$ 0.3 (6)	13.0 $\pm$ 0.2 (6)	
—	—	—	6.2 $\pm$ 0.1 (5)	>90	>90
—	—	+	4.3 $\pm$ 0.1 (5)		
—	+	+	4.3 $\pm$ 0.2 (5)		
Corticosterone (0.1 mM)					
—	—	—	6.3 $\pm$ 0.1 (5)	>90	>90
—	—	+	4.6 $\pm$ 0.1 (5)		
—	+	+	4.5 $\pm$ 0.2 (5)		

and de Nicola *et al.* 1968a). It is the inhibition of transport in response to ACTH (de Nicola *et al.* 1968a) or 3':5'-cyclic AMP (Table 5) that requires protein synthesis.

Tissue specificity. Thus far, all our data are consistent with the conclusion that the corticoids produced in response to ACTH or 3':5'-cyclic AMP cause the inhibition of ascorbate transport. If this response is peculiar to the adrenal gland and is the basis of the 'depletion' phenomenon *in vivo*, corticoids should inhibit ascorbate transport only in the adrenal gland and not in other tissues that are capable of transporting ascorbate.

The rat pituitary gland transports ascorbate by a mechanism similar to that observed in the adrenal gland (de Nicola *et al.* 1968b). Table 6 shows that ascorbate uptake in the pituitary is not affected by corticosterone or 3':5'-cyclic AMP, which have marked effects on the transport in the adrenal gland.

Glycine uptake in the pituitary gland is also unaffected by a variety of steroids (Table 6). This

is not because the steroids fail to penetrate since amino acid incorporation into proteins is inhibited by the corticoids in the pituitary gland. It is known that corticoids also inhibit amino acid incorporation into adrenal-gland proteins *in vitro* (Ferguson, Morita & Mendelsohn, 1967; Morrow, Burrow & Mulrow, 1967; Clayman *et al.* 1970). It may be noted that the pituitary gland is very active in accumulating amino acids such as glycine, serine and taurine. Concentration ratios of 17 and 30 are obtained with 1 μ M-serine and 0.1 mM-aurine respectively after 2 h of incubation.

Net uptake of ascorbate. One of the major differences between the estimates of ascorbate 'depletion' *in vivo* and the measurements of ascorbate transport *in vitro* is that changes in concentration of ascorbate are measured in the former whereas changes in concentration of radioisotope are measured in the latter. Since it is conceivable that the measurements *in vitro* might reflect an exchange process rather than a change in the absolute amount of ascorbate in the gland, we have

Table 6. *Corticoids and transport of glycine and ascorbate in the rat pituitary gland*

Conditions of incubation were as described for Table 1. Glycine concentration was 10 μ M, ascorbate 0.1 mM. Details of specific radioactivity and 14 C-labelled compounds used are given in Tables 1 and 3 respectively. The results are given as means $\pm$ S.E.M. with the numbers of experimental values in parentheses.

Substance tested	Additions	Uptake	$10^{-5} \times$ Amino acid incorporation (c.p.m./100 mg of protein)
		($\frac{\text{c.p.m./ml of cell water}}{\text{c.p.m./ml of medium}}$)	
Glycine	None	16.2 ± 0.9 (5)	2.2 ± 0.15 (5)
	Cholesterol (0.1 mM)	18.0 ± 1.3 (5)	2.3 ± 0.13 (5)
	Pregnenolone (0.1 mM)	17.3 ± 0.9 (5)	2.3 ± 0.15 (5)
	Corticosterone (0.1 mM)	16.8 ± 0.1 (5)	1.0 ± 0.05 (5)
	Corticosterone (0.5 mM)	17.3 ± 0.1 (5)	0.43 ± 0.01 (5)
	Aldosterone (0.1 mM)	16.8 ± 1.3 (5)	1.2 ± 0.11 (5)
	Aldosterone (0.5 mM)	17.6 ± 0.6 (5)	0.49 ± 0.01 (5)
Ascorbate	None	5.3 ± 0.4 (5)	—
	Corticosterone (0.1 mM)	5.8 ± 0.2 (4)	—
	3':5'-Cyclic AMP (10 mM)	5.4 ± 0.2 (5)	—

Table 7. *Ascorbate uptake*

Conditions of incubation were as described for Table 2. The results are given as means $\pm$ S.E.M., with the numbers of experimental values in parentheses. The statistical significance was determined in a paired Student's *t* test.

Additions	Conditions	Ascorbate uptake (μ mol/ml of cell water)	
I None 2 mM-Ascorbate 2 mM-Ascorbate + 0.1 mM-corticosterone	O ₂ (normal)	2.3 ± 0.21 (4)	$P < 0.05$
	O ₂ (normal)	4.8 ± 0.20 (4)	
	O ₂ (normal)	3.6 ± 0.33 (4)	
II 5 mM-Ascorbate 5 mM-Ascorbate 5 mM-Ascorbate + 0.1 mM-corticosterone	N ₂ (sucrose medium)	2.8 ± 0.11 (4)	$P < 0.01$
	O ₂ (normal)	8.0 ± 0.14 (4)	
	O ₂ (normal)	5.9 ± 0.28 (4)	

recently verified that the amount of ascorbic acid taken up by the adrenal gland *in vitro* from a fixed external concentration requires metabolic energy and Na⁺ ions. Further, the extent of uptake under aerobic conditions is decreased by the presence of corticosterone (Table 7). It should be pointed out that 10mm-3':5'-cyclic AMP acts in a similar manner to corticosterone and that without the addition of ascorbate to the incubation medium the concentration of ascorbate retained in the gland after 2 h of incubation is usually about twice as high under aerobic as under anaerobic conditions in Na⁺-free media. Therefore on the basis of tissue, substrate and hormonal specificity, these data support the conclusion that ascorbate 'depletion' *in vivo* is a consequence of a specific inhibition of ascorbate transport into the adrenal gland and that the reason the ascorbate-'depletion' assay for ACTH parallels other assays for ACTH based on steroid production is because the 'depletion' assay is actually dependent on the corticoids produced.

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