CHAPTER 5

MECHANISM OF CONVERSION OF D-GLUCURONOLACTONE TO L-ASCORBIC ACID : EFFECT OF SOME ALDEHYDE AGENTS

It was reported earlier (46, 50) that for the enzymic conversion of D-glucuronolactone to L-ascorbic acid, a high concentration of potassium cyanide was needed and cyanide could not be replaced by any other metabolic inhibitor or reducing agents (91). Throughout the present investigation also a rather high concentration of potassium cyanide has been used to study the *in vitro* conversion of D-glucuronolactone to L-ascorbic acid. However, recent studies with rat and goat liver microsomes (96) indicate that cyanide is not specific for this conversion. It can be replaced by other aldehyde agents viz. semicarbazide and hydroxylamine. It was, therefore, considered appropriate to see the effect of these aldehyde agents on the conversion of D-glucuronolactone into L-ascorbic acid by microsomes from different species of animals. Table XXXIII shows that irrespective of the species studied and irrespective of whether the enzyme is present in the kidney or the liver, the microsomes from all the species examined including the birds, can effect the conversion of D-glucuronolactone into L-ascorbic acid in the presence of semicarbazide and hydroxylamine. Table XXXIII further shows that the species which have been shown incapable of synthesizing the vitamin in presence of cyanide are also incapable of doing so when cyanide has been replaced by semicarbazide and hydroxylamine.

Table XXXIII

Effect of aldehyde agents on the conversion of D-glucuronolactone into L-ascorbic acid by microsomes from different species of animals

The incubation medium contained 0.25 ml microsomes, 0.02 M sodium phosphate buffer, pH 7.2, 0.005 M NaPPi, 5 mg of D-glucuronolactone and any one of 0.02 M KCN, 0.02 M $NH_2NHCONH_2$ or 0.01 M NH_2OH. When NH_2OH was used as the aldehyde agent, pH of the medium was 6.5. Incubation period was 2 hrs. at 37°.

Animals	Addition	Ascorbic acid (µg/mg protein) Kidney	Liver
Amphibian			
Toad	KCN	80.0	nil
	$NH_2NHCONH_2$	78.0	"
	NH_2OH	85.0	"
Reptile			
Tortoise	KCN	83.0	"
	$NH_2NHCONH_2$	82.0	"
	NH_2OH	85.0	"
Mammal			
Goat	KCN	nil	50.0
	$NH_2NHCONH_2$	"	50.0
	NH_2OH	"	70.0

(Continued)

Table XXXIII (Contd.)

Animals	Addition	Ascorbic acid (µg/mg protein) Kidney	Liver
Rat	KCN	nil	30.0
	$NH_2NHCONH_2$	"	32.0
	NH_2OH	"	35.0
Guinea pig	KCN	"	nil
	$NH_2NHCONH_2$	"	"
	NH_2OH	"	"
Birds			
Duck	KCN	70.0	"
	$NH_2NHCONH_2$	69.0	"
	NH_2OH	72.0	"
Pigeon	KCN	53.0	"
	$NH_2NHCONH_2$	55.0	"
	NH_2OH	58.0	"
Common myna	KCN	15.0	28.0
	$NH_2NHCONH_2$	13.0	31.0
	NH_2OH	18.0	31.0
Sparrow	KCN	nil	50.0
	$NH_2NHCONH_2$	"	53.0
	NH_2OH	"	55.0
Sun bird	KCN	"	nil
	$NH_2NHCONH_2$	"	"
	NH_2OH	"	"
Jungle babbler	KCN	"	28.0
	$NH_2NHCONH_2$	"	30.0
	NH_2OH	"	31.0
Paradise fly catcher	KCN	"	nil
	$NH_2NHCONH_2$	"	"
	NH_2OH	"	"

(Continued)

Table XXXIII (Contd.)

Animals	Addition	Ascorbic acid ($\mu g/mg$ protein) Kidney	Liver
Red vented bulbul	KCN	nil	nil
	$NH_2NHCONH_2$	"	"
	NH_2OH	"	"
Redwhiskered bulbul	KCN	"	"
	$NH_2NHCONH_2$	"	"
	NH_2OH	"	"
Grey shrike	KCN	"	"
	$NH_2NHCONH_2$	"	"
	NH_2OH	"	"

Inhibitory role of some aldehydes on the microsomal conversion of D-glucuronolactone into L-ascorbic acid

More recent studies in this laboratory indicate that the microsomal conversion of D-glucuronolactone into L-ascorbic acid is significantly inhibited by a number of aldehydes, the inhibition being reversed by a corresponding increase in the concentration of any of the aforesaid aldehyde agents used (Table XXXIV).

Table XXXIV

Effect of some aldehydes on the conversion of D-glucuronolactone into L-ascorbic acid

The incubation system contained 0.25 ml of goat liver microsomes (5 mg protein), 0.02 M sodium phosphate buffer, pH 7.2 and 5 mg D-glucuronolactone in a total volume of 2.5 ml; incubated for 2 hrs. at 37°. L-ascorbic acid was estimated titrimetrically.

Additions (µmole)	KCN (M)	L-ascorbic acid synthesized µg/mg. protein
None	-	nil
None	0.02	50
Formaldehyde (2.5)	0.02	20
" (2.5)	0.04	47
Acetaldehyde (2.5)	0.02	27
" (2.5)	0.04	47
Glycolaldehyde (2.5)	0.02	33
" (2.5)	0.04	47
Glyceraldehyde (10)	0.02	31
" (10)	0.04	48
Gluteraldehyde (2.5)	0.02	27
" (2.5)	0.04	47

Aldehyde function of D-glucuronolactone in solution

It has been observed that an increase in the concentration of D-glucuronolactone in the incubation medium results in an inhibition in the synthesis (Table XXXV). However, as has been mentioned before, the inhibition is reversed by a corresponding increase in any of the aforesaid aldehyde agents (Table XXXV). Contrary to the assumption that hexose sugars exist exclusively in the cyclic form, it has been observed that a freshly prepared solution of D-glucuronolactone contains a significant amount of the free aldehydo form as evident from its restoration of the color of Schiff's reagent and reduction of Fehling's solution at the room temperature, the reactions not responded to by D-glucose or sodium salt of D-glucuronic acid. This would indicate that probably the aldehydo form of D-glucuronolactone acts as a non competitive inhibitor to the enzyme and unless it is blocked by aldehyde agents D-glucuronolactone is not converted to L-ascorbic acid.

Table XXXV

Effect of increasing concentration of D-glucuronolactone on the synthesis of L-ascorbic acid by goat liver microsomes

The incubation media contain 0.25 ml of goat liver microsomes (5 mg protein), 0.02 M sodium phosphate, pH 7.2 in a total volume of 2.5 ml. Incubation period was 2 hrs. at 37°.

D-Glucuronolactone (μmole)	KCN (μmole)	L-ascorbic acid synthesized (μg/mg protein)
25	None	Nil
25	0.02	50
50	0.02	41
50	0.04	53
75	0.02	35
75	0.06	55
