Distribution data

Table I (b) shows that the activity of a homogenate resided almost entirely in the supernatant fraction. Other experiments summarized in Table I (c) again show that there is relatively little activity in the nuclear fraction as now prepared. This conclusion has been confirmed in further experiments (performed at the suggestion of Dr. V. R. POTTER; not tabulated) in which it was sought to damage the nuclei, by freezing or by exposure to distilled water.

The finding that activity is particularly high in supernatant fractions and low in nuclear fractions is not necessarily incompatible with the above-mentioned experiments[1–4], which were essentially qualitative in character and in which large amounts of catabolic products, such as UMP, were found. However, in view of the failure of MILLS and collaborators[1] to find pyrophosphorolysis of UDP-glucose to UTP with supernatant fractions, the possibility cannot be ruled out that there may be an enzyme which can form UDP-glucose from UTP but not UTP from UDP-glucose, as may be the case with the formation of UDP-acetylglucosamine from UTP[4]. A further possibility is that a uridine compound other than UTP is also capable of acting as a uridyl donor, as suggested by the finding[10] that shortly after injection of labelled orotic acid the labelling of UTP may be lower than that of UDP-glucose.

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On the microsomal and soluble lactonases

It is now clearly established that L-ascorbic acid is synthesized from D-glucuronic acid or D-glucuronolactone in animal tissues. Synthesis of ascorbic acid via L-gulonic acid and 3-keto-L-gulonic acid was proposed by LEHNINGER and his co-workers[1–3]. They

Abbreviations: DPN and TPN, di- and tri-phosphopyridine nucleotide; ATP, adenosine triphosphate.

demonstrated the synthesis of L-ascorbic acid from L-gulonic acid using extracts of the liver of some mammals and extracts of avian kidney, in the presence of DPN and Mn^{++}. However, in man, the monkey and the guinea pig, ascorbic acid could not be synthesized in the same system, although, DPN- and TPN-specific L-gulonic acid dehydrogenases are widely distributed in the liver and kidney of the animals so far tested and are not specific to organs or species synthesizing ascorbic acid. The specific deficiency of an enzyme or enzyme system in the man, monkey, and guinea pig has not hitherto been demonstrated.

The specific absence and abnormality of an enzyme lactonase in these animals is herein described and its possible role in ascorbic acid biosynthesis is discussed.

The enzymic hydrolysis of D-glucuronolactone and L-gulonolactone was previously described by HASSAN AND LEHNINGER in rat-liver extracts[1]. Studies in this laboratory on the distribution of lactonase activity in cell fractions showed the presence of two distinct lactonases, tentatively named lactonase-I and -II for soluble and microsomal fractions, respectively. These two enzymes differ in their substrate specificity and alkali lability. Lactonase-I has been partially purified from beef-liver acetone powder[4]. This enzyme shows rather broad substrate specificity (Table I), whereas lactonase-II does not act on D- and L-gulonolactone, D-mannonolactone, L-galactonolactone, and D-glucono-δ-lactone.

TABLE I

SUBSTRATE SPECIFICITY OF LACTONASE-I

Substrate (γ-lactone)	*Activity (%)*	*Substrate (γ-lactone)*	*Activity (%)*
L-Gulono-	100	D-Glucurono-	31
D-Gulono-	278	D-Idono-	0
L-Galactono-	139	D-Glucono-δ-	40
D-Galactono-	160	D-Glucuronic acid, ethyl ester	0
L-Glucono-	20	Triacetin	0
D-Glucono-	190	Glycerophosphate	0
D-Mannono-	31		
D-Mannurono-	75		

Lactonase activity was measured manometrically as CO_2 output from $NaHCO_3$ buffer (pH 7.2). Each flask contained 0.4 ml 0.1 *M* $NaHCO_3$, 0.1 ml 0.1 *M* $MgSO_4$, 0.3 ml 10^{-3} *M* glutathione, and 1.0 ml of enzyme solution in the main chamber, and 30 μmoles of lactone in 0.3 ml of water in the side arm; total vol., 3.0 ml; gas phase, 6% CO_2, 94% N_2. The reaction was started by tipping in the substrate after temperature equilibration to 37°.

These findings, showing the diversity and rather complex features of lactone hydrolysis, were interesting in connection with the problem of ascorbic acid biosynthesis, and further studies on the distrubution of these two lactonases in a variety of animals were made. The results of such an examination are summarized in Table II, from which the following conclusions may be drawn. (i) The presence of the lactonases was shown in mammalian liver and avian kidney. (ii) Lactonase-II was present in all the species tested. (iii) Lactonase-I was found only in those organs in which L-ascorbic acid could be synthesized from L-gulonic acid. The liver of man and monkey completely lacked this enzyme. In the case of the pigeon, it was found in the kidney, but not in the liver. (iv) The guinea-pig liver was peculiar in that, although lactonase was present in the supernatant fraction, its substrate specificity was different from that of

TABLE II

DISTRIBUTION OF LACTONASES

Species	Tissue	Mitochondria		Microsomes		Supernatant	
		D-glucurono-	L-gulono-	D-glucurono-	L-gulono-	D-glucurono-	L-gulono-
Ox	liver	0	0	11.8	0	90	277
Rat	liver	0	0	6	0	80	263
Rabbit	liver	0	0	15.0	0	26	63
	kidney	0	0	0	0	0	0
Pigeon	liver	0	0	0	0	0	0
	kidney	0	0	1.1	2.4	35	120
Guinea pig	liver	0.6	0	5.6	0	56	67
Monkey*	liver	0	0	0.6	0	0	0
Man	liver	0	0	6.2	0	0	0

Conditions as Table I. All activities are expressed as μmoles lactone hydrolysed/1 g wet tissue during the initial 10 min. Each cell fraction was prepared according to SCHNEIDER AND HOGEBOOM[7]. Owing to the alkali lability of the microsomal enzyme, the microsomal suspension was added to the reaction mixture immediately before the gas exchange.

* *Maccac fuscata* Y.

the usual lactonase-I. L-Gulonolactone and D-glucuronolactone were hydrolyzed at almost the same rate. Thus, the guinea-pig-liver lactonase acts more sluggishly on L-gulonolactone than the usual lactonase-I.

These results suggest that lactonase-I has an important role in ascorbic acid biosynthesis. Other studies in this laboratory with purified DPN-linked L-gulonic acid dehydrogenase from guinea-pig liver failed to show the formation of 3-ketogulonic acid as the reaction product[5]. The decarboxylation of the proposed ketohexonic acid could not be separated from the dehydrogenase activity. On the other hand, BURNS reported that rat-liver microsomes converted L-gulonolactone to L-ascorbic acid with considerable efficiency[6], and the presence of an enzyme which acted only on gulonolactone in the presence of 2,6-dichlorophenolindophenol was also found in this laboratory. Because the reversal of the lactonase reaction was demonstrated to some extent with L-gulonic acid, the above results might suggest that L-gulonolactone is the most probable immediate precursor of ascorbic acid. Further studies of lactonization and its physiological significance are now under investigation.

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