Table 2. Conversion of L-gulonolactone to L-ascorbic acid in rat and guinea pig livers. Five milligrams of L-gulonolactone-1-C^{14} (0.6 μc) was incubated for 90 minutes at 37°C under air in 5 ml of a 10-percent homogenate (or microsomes from an equivalent amount of liver) in 0.15*M* phosphate buffer (*p*H 7.20) containing 0.13*M* sucrose. L-Ascorbic acid was isolated after addition of carrier (150 mg) from a 5-percent trichloroacetic acid extract of the incubation mixture as its 2,4-dinitrophenylosazone derivative (*3*).

Species	Conversion (%)
Homogenate	
Rat	12.0
Rat	8.0
Rat	3.6
Guinea pig	< 0.05
Guinea pig	< 0.05
Microsomes	
Rat	18.6
Rat	7.7
Rat	3.8
Guinea pig	< 0.05
Guinea pig	< 0.05

why these species are unable to synthesize L-ascorbic acid. This report presents results that point out a possible missing biochemical step in guinea pigs required for the synthesis of the vitamin.

Previous work has shown that L-ascorbic acid is formed from D-glucose in the rat by the following steps: D-glucose → D-glucuronolactone → L-gulonolactone → L-ascorbic acid. Evidence for this scheme comes from experiments in which the incorporation of C^{14} into L-ascorbic acid was measured after administration of various D-glucose tracers (*1*), uniformly labeled D-glucuronolactone (*2*) and carboxyl-labeled D-glucuronolactone and L-gulonolactone (*3*). Further evidence for this pathway in rats was presented by Isherwood and coworkers (*4*), who reported increased urinary excretion of L-ascorbic acid after administration of nonlabeled D-glucuronolactone and L-gulonolactone.

In the present study the role of L-gulonolactone as a precursor for L-ascorbic acid biosynthesis in rats and guinea pigs was compared. In contrast with the appreciable conversion of L-gulonolactone to L-ascorbic acid in the rat, no detectable conversion was observed in guinea pigs (Table 1). Similarly, results of experiments *in vitro* showed considerable synthesis of L-ascorbic acid from L-gulonolactone in homogenates of rat liver but none in those of the guinea pig (Table 2) (*5*). Cellular fractionation studies (*6*) showed that the conversion of L-gulonolactone to L-ascorbic acid occurs chiefly in the microsomes of rat liver, but no activity was detected in the microsomes of guinea pig liver (Table 2). Rat liver mitochondria possess about one-fourth of the activity of microsomes in converting L-gulonolactone to L-ascorbic acid, but none was found in the mitochondria of the guinea pig liver. The system for converting L-gulonolactone to L-ascorbic acid in the rat is apparently present only in the liver, since incubation of L-gulonolactone-1-C^{14} under identical conditions in homogenates of kidney, brain, small intestines, and muscle resulted in no synthesis of the vitamin (< 0.05-percent conversion).

These results showing that microsomes of guinea pig liver are unable to convert L-gulonolactone to L-ascorbic acid point out the possible missing biochemical step in this species needed for the synthesis of L-ascorbic acid. A more definite conclusion on whether this is the only step missing in guinea pigs will be possible when information becomes available on the enzymes required for the synthesis of L-gulonolactone from D-glucose.

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5. Unpublished experiments with L-ascorbic acid-1-C^{14} show that this compound is extensively metabolized in rat liver homogenates but to a lesser extent in guinea pig homogenate. The values reported in Table 2, therefore, represent minimum values for the conversion of L-gulonolactone to L-ascorbic acid in the rat liver.
6. Microsomes and mitochondria were prepared in isotonic sucrose by the method of W. C. Schneider and G. H. Hogeboom [*J. Biol. Chem.* **183**, 123 (1950)].

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Table 1. Accumulation of uric acid and its precursors (hypoxanthine and xanthine) in rat liver as a result of anaerobiosis. Incubation was carried out for 1 hour at 37.5°C in 100-percent oxygen or nitrogen.

Fraction	Uric acid	Hypoxanthine + xanthine
	(μmole/100 g wet weight of liver or equivalent of incubation medium)	
Incubation in oxygen		
Tissue	8.1	4.8
Medium	0.0	21.5
Total	8.1	26.3
Incubation in nitrogen		
Tissue	14.5	62.5
Medium	72.7	240.0
Total	87.2	302.5

Uric Acid Metabolism and the Mechanism of Iron Release from Hepatic Ferritin

Previous studies in our laboratory have suggested that lowered oxygen tension is a stimulus for the release of iron from ferritin stores in the liver. During the hypoxia that attends the drastic hypotension of hemorrhagic shock, increasing quantities of iron appear in the plasma, in amounts almost sufficient to saturate the iron-binding capacity of the plasma. *In vitro* studies have shown that, as ferritin iron is reduced to the ferrous state, it becomes less tightly bound to the protein and can transfer across a membrane or be removed from ferritin in the presence of an avid iron-binding compound such as α,α′-dipyridyl or the iron-binding protein of the plasma (*1*).

The results of the present study (*2*) point to the involvement of the enzyme, xanthine oxidase, as well as the product of its activity, uric acid, in the reduction of ferritin iron. Slices of normal rat liver that are incubated in a nitrogen atmosphere in Ringer-phosphate solution liberate into the medium a substance that is capable of reducing ferritin iron. This compound has been identified as uric acid by means of its absorption spectrum, reduction of the uric acid reagent, and the disappearance of these two properties after incubation with the enzyme, uricase, which is highly specific for uric acid.

Analyses of both the liver slices and the medium in which they had been incubated (Table 1) revealed an over-all accumulation of uric acid. Of the rat tissues examined, liver, spleen, kidney, and small intestine, only the liver produced more uric acid in response to lowered oxygen tension; the others produced less. An examination of aerobic and anaerobic liver slices for their content of uric acid precursors, xanthine and hypoxanthine, showed a considerable accumulation of these compounds under anaerobic conditions (Table 1). This increase is presumed to result from depletion of high-energy compounds needed for synthetic reactions involving the purines, and from the resultant increase in catabolic reactions (*3*). Uricase, which is present in rat liver, was found to be much more sensitive to inhibition by lowered oxygen tension than xanthine oxidase.

Xanthine oxidase may act as a dehydrogenase in the presence of a suitable

acceptor, such as methylene blue or cytochrome *c* (*4*). The possibility was explored that ferritin might be reduced as a result of xanthine dehydrogenase activity under conditions of lowered oxygen tension. When ferritin was added to an anaerobic mixture of xanthine oxidase, hypoxanthine, or xanthine, and α,α′-dipyridyl to act as a trapping agent, the ferrous iron content of the system was increased 2.6 times. In this anaerobic reaction, the first reduction of ferritin iron must occur by virtue of its action as an electron acceptor, for no uric acid is formed in the absence of ferritin.

The reduction of inorganic iron by xanthine oxidase has been reported by Weber *et al.* (*5*) to be dependent on the formation of H_2O_2. The presence of oxygen in our system stimulated reduction of ferritin iron. This may be explained by the formation, in oxygen, of increased amounts of uric acid, which reduces more ferritin. The addition of crystalline catalase to the aerobic reaction mixture caused a further stimulation of ferritin reduction, presumably by protecting the enzyme against inactivation by H_2O_2. Therefore, H_2O_2 is apparently not involved in the reduction of ferritin iron.

A homeostatic mechanism for the regulation of plasma iron levels is suggested by these results. In addition, preliminary experiments indicate the presence in aerobic liver of a system (as yet uncharacterized) that is capable of oxidizing ferrous ferritin to the ferric state. In the normal aerobic liver, ferrous ferritin, formed as a result of the activity of xanthine oxidase on relatively low levels of hypoxanthine and xanthine, would be largely reoxidized. In the hypoxic liver, as a consequence of dehydrogenase activity on increased quantities of hypoxanthine and xanthine, more ferritin is reduced to the ferrous form and cannot be reoxidized. This results in increased levels of plasma iron.

In vivo confirmation of the role of uric acid in the release of ferritin iron to the plasma has been obtained from preliminary experiments carried out with S. Baez and S. G. Srikantia in our laboratory, using rats subjected to hemorrhagic shock. In such animals, we observe an increased plasma uric acid level, which cannot be accounted for solely by inhibition of kidney function caused by lowered blood pressure. Similar increases in plasma uric acid have been reported by others (*6*) without explanation of the mechanism involved.

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Criteria for Assessing Effects of Drugs on Posttetanic Potentiation

There is considerable evidence that posttetanic potentiation (PTP) is a general synaptic property and is a normal consequence of synaptic excitation (*1–5*). Modification of the potentiating process by drugs (for example, *6–8*) provides clues to the nature of the phenomenon as well as information concerning the mechanisms of action of the drugs. Because of anatomical and physiological limitations on size of the efferent neuronal pool (in monosynaptic pathways of the spinal cord and in sympathetic ganglia), it is possible in some circumstances to modify profoundly the potentiating process without affecting the degree of PTP of synaptic transmission as tested in the usual manner, namely, by comparison of maximal synaptic discharges before and after tetanic stimulation. Jefferson and Benson (*9*) have shown that under certain conditions the total relevant motoneuron pool may be discharged during maximum PTP of the monosynaptic (2N) pathway of the spinal cord. Furthermore, at high levels of excitability such potentiation may be more than sufficient to lead to excitation of available motoneurons.

Figure 1*A* shows input-output relations in the 2N pathway of the spinal cord in the resting state (*10*) and during the period of maximum PTP (*11*) at various levels of reflex excitability as controlled by spinal cord temperature (*12*). With increasing size of maximum discharges at rest (*Y* intercepts of curves *a, b, c,* and *d*) the maximum potentiated discharges increase to a ceiling (curves *c′* and *d′*) imposed by limitations on available postsynaptic neurons. Curves representing PTP with time after tetanus at the corresponding levels of excitability show that the maximum posttetanic discharges must be the same for all curves (*c′ c″*; *d′, d″*) on this ceiling.

Therefore, when the input-output

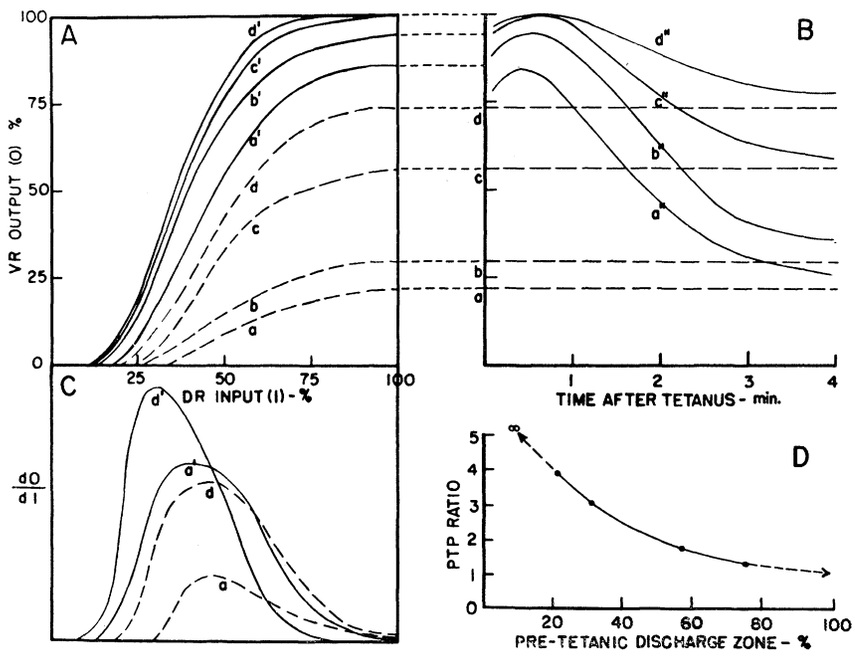

Fig. 1. Various aspects of PTP in the monosynaptic pathway of the spinal cord studied at four levels of excitability. (*A*) Experimentally determined input-output curves at rest (*a–d*) and during the peak of PTP (*a′–d′*). Excitability was altered by varying spinal cord temperature. Temperatures for curves *a–d* were 36°C, 35°C, 32°C, and 30°C, respectively. Unanesthetized spinal cat, DR-VR (S_1) preparation. (*B*) Curves illustrating time-course of PTP at the various levels of excitability. (*C*) Curves illustrating distributions of synaptic thresholds for several of the curves in *A*, obtained by graphical differentiation of corresponding input-output curves. Areas under curves are proportional to respective discharge zones in *B*. (*D*) relationship between PTP ratio and discharge zone at rest. Values plotted are those obtained from the *Y* intercepts of *A*.