fractions by the method of Crane. Brush border membrane contained most of the mucosal invertase but little of the mutarotase. Ninety per cent of the mutarotase remained in a supernatant fraction after removal of brush border. 4. The possible implications of these findings in connection with mechanism of sugar transport are discussed.

1. Keilin, D., Hartree, E. F., *Biochem. J.*, 1951, v50, 341.
2. Keston, A. S., *Fed. Proc.*, 1958, v253, 999.
3. ———, *Science*, 1954, v120, 355.
4. Crane, R. K., Krane, S. M., *Biochim. et Biophys. Acta*, 1956, v20, 568.
5. Wilson, T. H., Landau, B. R., *Am. J. Physiol.*, 1960, v198, 99.
6. Bailey, J. Martyn, Pentchev, Peter, *Biochem. Biophys. Res. Comm.*, 1964, v14, 161.
7. McDougal, D. B., Jr., Little, K. D., Crane, R. K., *Biochim. et Biophys. Acta*, 1960, v45, 483.
8. Miller, D., Crane, R. K., *Anal. Biochem.*, 1961, v2, 1.
9. Borgstrom, B., Dahlqvist, A., *Acta Chem. Scand.*, 1958, v12, 1997.
10. Gallo, L. L., Treadwell, C. R., PROC. SOC. EXP. BIOL. AND MED., 1963, v114, 69.
11. Keston, A. S., *Fed. Proc.*, 1955, v14, 234.

Received November 14, 1963. P.S.E.B.M., 1964, v115.

Effects of Hormones and Drugs on Activity of L-Gulono-γ-Lactone Hydrolase.* (29042)

LOTHAR L. SALOMON, DONALD W. STUBBS AND HATIM F. DAGINAWALA
Department of Biochemistry and Nutrition, University of Texas Medical School, Galveston

It has been established that the synthesis of ascorbic acid by rats is diminished following hypophysectomy without concomitant decrease in formation of D-glucuronic acid or activities of L-gulonate NADP oxidoreductase, D-glucurono-δ-lactone hydrolase and L-gulono-γ-lactone oxidase(1,2,3). The concentration of $NADPH_2$ actually increased(1). Therefore, it has been suggested that the lowered rate of synthesis of ascorbic acid may be ascribed to the depressed activity of L-gulono-γ-lactone hydrolase(2,3). In agreement with this view was the finding that replacement therapy with somatotropin led to marked increases in the activity of L-gulono-γ-lactone hydrolase and the synthetic ability (3). But the production of D-glucuronic acid, and the activity of enzymes other than L-gulono-γ-lactone hydrolase which are responsible for its conversion to ascorbic acid, remained unaffected.

The sequence in which changes in activity of L-gulono-γ-lactone hydrolase and concentrations of ascorbic acid in serum and urine occur following daily administration of somatotropin to hypophysectomized rats has now been studied. The evidence corroborates the view that somatotropin influences the synthesis of ascorbic acid through control of the activity of L-gulono-γ-lactone hydrolase. Although the mechanism whereby somatotropin affects this enzymic activity is not understood, it may be assumed that the hormone stimulates the anabolism of proteins. However, it is doubtful that insulin is essential for the effect of somatotropin on hepatic L-gulono-γ-lactone hydrolase, since the activity of this enzyme remains at normal level in rats made severely diabetic with alloxan. A joint requirement for these two hormones in striated muscle has been observed(4). It is of interest in this connection that DL-ethionine produces changes in the system under investigation which closely mimic those observed after hypophysectomy of long standing.

It is also found that, of 2 possible alternate pathways, the route which circumvents the step catalyzed by D-glucurono-δ-lactone hydrolase must be quantitatively far more important in the synthesis of ascorbic acid. The reported inhibitory effect of Chloretone

* This investigation was supported by research grant from Nat. Cancer Inst., Public Health Service.

TABLE I. Effect of Somatotropin on L-Gulono-γ-lactone Hydrolase and Ascorbic Acid in Serum and Urine of Hypophysectomized Rats.

			L-Gulono-γ-lactone Hydrolase				
No. of injections*	0	1	3	5	8	11	14
Mean activity†	1.0	1.2	1.7	1.9	2.0	2.2	2.3
S.D. and No. of exp	.2 (3)	.1 (2)	.0 (2)	.1 (2)	.2 (2)	.1 (2)	.1 (3)
			Ascorbic Acid in Serum				
No. of injections*	0	3	6	8	12	14	
Mean concn (mg/100 ml)	.9	.9	1.1	1.2	1.2	1.3	
S.D. and No. of Exp	.1 (4)	.1 (4)	.0 (4)	.2 (5)	.1 (4)	.1 (5)	
			Ascorbic Acid in Urine				
No. of injections*	0	5	9	11	15		
Mean excretion (mg/kg/6 hr)	.8	.9	1.3	1.6	1.6		
S.D. and No. of exp	.1 (5)	.1 (5)	.2 (5)	.2 (5)	.3 (5)		
Mean wt (g)	105	126	135	142	158		

* Dose 300 γ of somatotropin NIH-GH-B_6 per day (1 unit/mg), intraperitoneally.
† Micromoles of L-gulono-γ-lactone hydrolyzed by nonparticulate fraction from 3.75 mg liver/hour(2).

on L-gulono-γ-lactone hydrolase(5) appears to be restricted to purified preparations of this enzyme, and thus does not contradict its proposed mechanism of action and role in the synthesis of ascorbic acid.

Methods and materials. Analytical procedures were described previously(1,2,3); minor modifications are indicated where appropriate. Similarly, details pertaining to experimental animals were as before, except that diabetic rats tended to be larger (initial weights 311, 378, 289 and 330 g; after treatment 244, 216, 276 and 316 g, respectively).

Diabetes was induced by administration of 9 mg of alloxan per 100 g of body weight, subcutaneously on 2 days following a period of fasting for 24 hours(6). Rats selected for this study exhibited "4+" glucosuria(7) and very marked hyperglycemia(8). Portions of the pancreas of these and control rats were fixed in Romeis' fluid(9), sectioned and stained(10).

DL-Ethionine was injected intraperitoneally in doses of 50 mg per animal. Increased mortality of hypophysectomized rats necessitated reduction of the dose to 25 mg per day and limitation of the duration of the experiment to 4 days.

Results and discussion. Considerable parallelism was noted between changes in synthesis of ascorbic acid by rats and activity of hepatic L-gulono-γ-lactone hydrolase following hypophysectomy and during replacement therapy with somatotropin(1,2,3). The absence of other demonstrable relevant changes, including those resulting from altered intake of food(11), suggested that the step catalyzed by L-gulono-γ-lactone hydrolase became limiting after hypophysectomy, leading to a defect in synthesis of ascorbic acid(2) which was partly reversible by somatotropin through hormonally-induced increase in activity of the deficient enzyme(3). In support of this, present data (Table I) show that the changes elicited by somatotropin occur in the required sequence, namely, the increase in activity of L-gulono-γ-lactone hydrolase precedes the rise in concentration of ascorbic acid in serum and this, in turn, the rise in urinary ascorbic acid. These data also demonstrate that this enhanced urinary excretion following daily administration of somatotropin cannot be the result of diminished renal reabsorptive capacity for ascorbic acid. The observed rise of the level in serum prior to that in urine occurs because of the partial threshold characteristics of ascorbic acid(12); no such rise would be expected if urinary changes were the result of changes in renal efficiency. It may be noted that, analogously, lowered synthesis was reflected by disproportionate decrease in urinary excretion(1).

The manner in which somatotropin controls the activity of L-gulono-γ-lactone hydrolase (and, through it, the rate of synthesis of ascorbic acid) is a problem of fundamen-

TABLE II. A. Effect of DL-Ethionine in Intact Rats.*

Ethionine, No. of injections	Enzymic activites: Gulonolactone hydrolase† (μmole/hr)	Glucuronolactone hydrolase‡ (μmole/hr)	Gulonate NADP oxidoreductase (μmole/20 min.)	Gulonolactone oxidase (μmole/hr/g)
0	4.0 ± .3 (6)	5.6 ± .9 (6)	.075 ± .004 (3)	4.7 ± .8 (6)
2	3.7 ± .4 (2)	5.5 (1)	.078 ± .008 (2)	4.8 (1)
4	1.9 ± 1.0 (2)	—	—	—
6	1.4 ± 1.3 (2)	5.0 (1)	.079 ± .000 (2)	4.8 (1)
8	1.7 ± 1.4 (2)	—	.034 (1)	—
10	1.6 ± 1.1 (2)	5.0 (1)	.035 ± .017 (2)	4.8 (1)
12	.9 ± .1 (2)	—	.018 (1)	—

B. Enzymic Activities 14 Months after Hypophysectomy.

0	1.4 ± 1.3 (4)	5.1 ± .1 (4)	.053 ± .02 (4)	5.0 ± .3 (4)

C. Gulonolactone Hydrolase in Alloxan Diabetes.

Days after alloxan	Gulonolactone hydrolase (μmole/hr)	Serum glucose (mg/100 ml)
10	4.6 ± .2 (2)	437 ± 173 (2)
16	4.2 ± .1 (2)	665 ± 177 (2)

D. Effect of Chloretone and Chloral Hydrate on Gulonolactone Hydrolase.

Additions	Reaction mixture: L-Gulono-γ-lactone: Non-particulate fraction (μmole/hr)	L-Gulono-γ-lactone: Enzyme§ 43 units (μmole/15 min.)	D-Galactono-γ-lactone: Enzyme§ 7.6 units (μmole/15 min.)
None	2.5	4.1	7.6
"	2.7		
Chloretone (70% satd)	2.5	2.8	3.2
Chloral hydrate .05 M	1.6	—	—
.005 M	2.3	—	—
.0005 M	2.3	—	—

* Enzymic activities reported in terms of substrate reacting during indicated period as means with S.D.; number of experiments in parentheses. Activity of L-gulono-γ-lactone hydrolase in hypophysectomized rats diminished from 1.9 μmole/hr to 1.5 μmole after 2 injections, and to 1.0 μmole (2 rats) after 4 injections.

† Substrate D-gulono-γ-lactone.

‡ There may be a trend toward decreased activity in the ethionine-treated group as well as in rats 14 months after hypophysectomy.

§ Enzymic activity = 5,500 units/mg protein; unit = quantity hydrolyzing 1 μmole D-galactono-γ-lactone under conditions described(2) with 0.07 M substrate (Salomon and Daginawala, unpublished).

tal interest. Prior evidence showed that not all enzymic activities declined after hypophysectomy(2); that of some adaptive enzymes increased(13). In general, however, it may be assumed that somatotropin exerts a stimulatory effect on protein anabolism. The mechanism of action of DL-ethionine is also not completely understood. It may interfere with protein anabolism by one or more possible modes, perhaps including an inhibitory effect on synthesis of somatotropin(14). Present work shows that DL-ethionine rapidly induces decrease in activity of L-gulono-γ-lactone hydrolase, as shown in Table II (footnote) even in hypophysectomized rats. This means that the effects of the inhibitor are not solely those involving the synthesis of somatotropin, which is not surprising. The drug also results in rapid decrease of L-gulono-γ-lactone hydrolase activity and a subse-

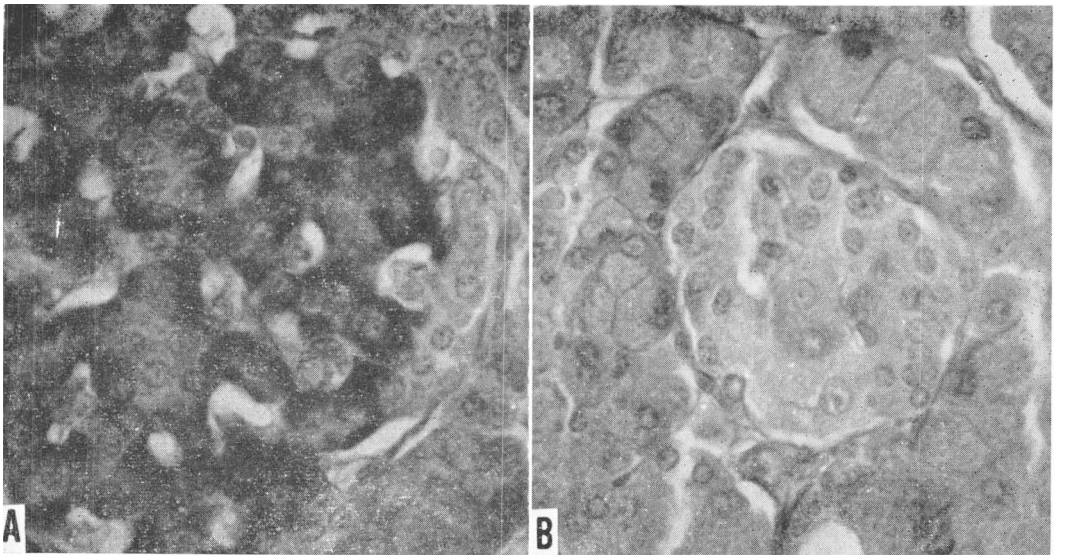

FIG. 1. Islet of normal (A) and alloxan-diabetic (B) pancreas of rats. Demonstrable beta granules characteristically absent in sections from diabetic rats, but prominent in normal islets. Sections processed simultaneously; about × 600; basic aldehyde-fuchsin (Gomori) with light green-orange G counterstain(10).

quent decrease in activity of L-gulonate NADP oxidoreductase in intact rats, but no appreciable changes occur during the experimental period in D-glucurono-δ-lactone hydrolase and L-gulono-γ-lactone oxidase (Table IIA). It is interesting that this is substantially the same situation which follows as a consequence of hypophysectomy. Although L-gulono-γ-lactone hydrolase quickly decreased in activity after removal of the pituitary gland(2), the latter two enzymes retain essentially full activity even 14 months after the operation (Table IIB). L-Gulonate NADP oxidoreductase remained unchanged for at least several weeks(2), but it must undergo gradual decline in activity since, after 14 months, it also is significantly depressed (Table IIB). Thus, removal of the endogenous source of somatotropin and administration of DL-ethionine lead to consequences which are quite similar in regard to enzymic effects and in the sequence in which these effects become evident, the action of the inhibitor being more rapid and profound.

The concerted action of somatotropin and insulin may be required for striated muscle *in vivo*(4). Evidence has now been obtained that somatotropin may be independent of insulin in the liver, since L-gulono-γ-lactone hydrolase retains normal activity in alloxan-diabetic rats (Table IIC). Although it is possible that such rats still produce small amounts of insulin, histological evidence indicates an absence of functional β-cells (Fig. 1), in agreement with work by others(9). Furthermore, others have shown insulin levels to be depressed sufficiently in alloxan diabetes to interfere with the action of somatotropin on striated muscle(4). This problem is under further investigation.

The importance of L-gulono-γ-lactone hydrolase in the pathway leading to ascorbic acid may reside in its ability to catalyze the interconversion of L-gulonic acid and its γ-lactone (Fig. 2)(15), or it may form gulonyl-enzyme complex from either L-gulonic acid or the lactone to provide substrate for L-gulono-γ-lactone oxidase in the form of the complex(5). Both mechanisms are consistent with the contention that the enzyme catalyzes an important step in the synthesis of ascorbic acid. It is possible to distinguish between the two alternatives by determining the relative efficiencies of L-gulonic acid and L-gulono-γ-lactone as precursors for ascorbic acid in preparations obtained from intact and

TABLE III. Synthesis *in Vitro* by Systems from Intact and Hypophysectomized Rats.

	From sodium L-gulonate asc. acid (γ/sample)*	From L-gulono-γ-lactone asc. acid (γ/sample)*
Intact	17.9 ± 1.4 (2)	44.4 ± 1.3 (2)
Hypophysectomized	10.9 ± 2.3 (3)	69.5 ± 13.1 (3)
Relative efficiency intact/ hypophysectomized	1.6	.6

Complete system equivalent to 120 mg liver incubated with 3 mole sodium L-gulonate or 3.4 μmole L-gulono-γ-lactone at 25° for 1 hr under O_2; other details as described for complete system(3). L-gulono-γ-lactone oxidase activity(3): Intact rats, 5.0 and 4.4 μmoles of ascorbic acid/g of liver/hr; hypophysectomized rats, 4.8, 4.7 and 4.7 μmoles/g/hr.

* S.D. and number of experiments are indicated.

hypophysectomized rats. Data summarized in Table III represent a comparison of such systems with nearly identical L-gulono-γ-lactone oxidase activity, but differing in activity of L-gulono-γ-lactone hydrolase. In the systems with low hydrolase activity, *i.e.*, those from hypophysectomized rats, L-gulono-γ-lactone is more efficiently oxidized to ascorbic acid. Conversely, L-gulonic acid is more efficiently converted to ascorbic acid by systems from intact rats. Taken together, this evidence indicates that L-gulonic acid improves as a precursor when the activity of L-gulono-γ-lactone hydrolase is high because more lactone is formed and made available to the oxidase. When lactone is the substrate, the higher the activity of the hydrolase, the more rapid will be the hydrolytic reaction leading to free acid; therefore, systems from hypophysectomized rats with low hydrolase activity form more ascorbic acid under these circumstances. This is not as predicted from consideration of the role of the gulonyl-enzyme complex(5), but consistent with the conclusions of Winkleman and Lehninger (15).

Another aspect of the foregoing results is that they permit estimation of the relative importance of the alternate routes from D-glucuronic acid to ascorbic acid (Fig. 2). If steps 3→4→5 were important quantitatively, it would have to be concluded that hypophysectomy should enhance, rather than

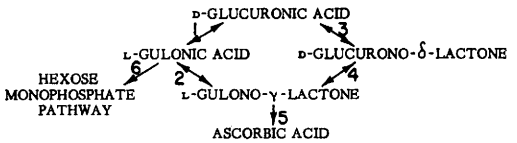

FIG. 2. Part of glucuronate pathway.

damage, the ability to synthesize ascorbic acid. (It is to be noted that the activity of D-glucurono-δ-lactone hydrolase is never seriously impaired, and that of L-gulonate NADP oxidoreductase not until at least weeks after the essentially immediate effect of hypophysectomy on synthetic ability.) This being contrary to fact, the sequence of steps 3→4→5 appears to have at most minor significance as against the sequence 1→2→5, and it is unlikely that the sequence 3→4→2→6 functions as a main route or bypass to the hexose monophosphate pathway.

Chloretone, a drug known to promote the synthesis of ascorbic acid(16,17), has been reported to depress the activity of L-gulono-γ-lactone hydrolase(5). However, this would require a more substantial role for D-glucurono-δ-lactone hydrolase in the synthesis of ascorbic acid than assigned on the basis of results discussed above. Earlier studies of rats treated with Chloretone *in vivo* showed the absence of any effect on L-gulono-γ-lactone hydrolase or several other enzymes(3). Present work demonstrates that even nearly saturated solutions of Chloretone added *in vitro* do not affect the hydrolase in a 100,000 × *g* supernate from liver, but purified preparations of the enzyme are inhibited (Table IID). Chloral hydrate, a weaker stimulant of ascorbic acid synthesis, said to have an effect similar to that of Chloretone on the hydrolase(5), does indeed inhibit the crude preparations, but only in concentrations which are unequivocally toxicological and probably unattainable *in vivo*. The greater sensitivity of purified enzymes to physical and chemical influences is not surprising. But it is doubtful whether physiological sig-

nificance can be attached to this effect of the hypnotic drugs. That the drugs have a site of action preceding D-glucuronic acid is more probable in the light of the present and previous data(3), and the elegant work of Hollmann and Touster(18).

Summary. The temporal sequence of changes in activity of L-gulono-γ-lactone hydrolase, in concentration of ascorbic acid in serum and in urine during replacement therapy of hypophysectomized rats with somatotropin was examined. Results agree with the view that variations in activity of the hydrolase are responsible for diminished synthesis of ascorbic acid after hypophysectomy and increased synthetic activity when somatotropin is administered. The prompt decrease in activity of the hydrolase found after removal of the hypophysis did not occur in severe alloxan diabetes, indicating that permissive action of insulin may not be required for effects of somatotropin on hepatic protein anabolism. However, DL-ethionine rapidly depressed the activity of the hydrolase and of L-gulonate NADP oxidoreductase in a way analogous to long-term consequences of hypophysectomy. It appears that an alternative to the step involving lactonization of L-gulonic acid cannot be quantitatively important for synthesis of ascorbic acid. Inhibition of the hydrolase by Chloretone and chloral hydrate *in vitro,* in apparent contradiction since these drugs stimulate biosynthesis of ascorbic acid, is probably of no relevance *in vivo.*

1. Salomon, L. L., Stubbs, D. W., *Ann. N. Y. Acad. Sci.,* 1961, v92, 128.
2. ———, *Biochem. Biophys. Res. Comm.,* 1961, v4, 239.
3. ———, *ibid.,* 1961, v5, 349.
4. Kipnis, D. P., Reiss, E., *J. Clin. Invest.,* 1960, v22, 409.
5. Isherwood, F. A., Mapson, L. W., Chen, Y. T., *Biochem. J.,* 1960, v76, 157.
6. Hall, J. C., *J. Biol. Chem.,* 1960, v235, 6.
7. Bray, W. E., *Clinical Laboratory Methods,* 1951, C. V. Mosby Co., St. Louis, 49.
8. Salomon, L. L., Johnson, J. E., *Anal. Chem.,* 1959, v31, 453.
9. Barrnett, R. J., Marshall, R. B., Seligman, A. M., *Endocrinology,* 1955, v57, 419.
10. Curr, E., *Methods of Analytical Histology and Histo-Chemistry,* 1959, Williams and Wilkins Co., Baltimore, pp. 57, 211.
11. Stubbs, D. W., Salomon, L. L., *Fed. Proc.,* 1963, v22, 422.
12. Friedman, C. J., Sherry, S., Ralli, E. P., *J. Clin. Invest.,* 1940, v19, 685.
13. Harding, H. R., Rosen, F., *Fed. Proc.,* 1963, v22, 409.
14. Stekol, J. A., *Advances in Enzymology,* 1963, v25, Nord, F. F., Ed., Interscience Publishers, New York, 369.
15. Winkleman, J., Lehninger, A. L., *J. Biol. Chem.,* 1958, v233, 794.
16. Longenecker, H. E., Fricke, H. H., King, C. G., *ibid.,* 1940, v135, 497.
17. Burns, J. J., Conney, A. H., Dayton, P. G., Evans, C., Martin, G. R., Taller, D., *J. Pharm. Exp. Therap.,* 1960, v129, 132.
18. Hollmann, S., Touster, O., *Biochim. Biophys. Acta,* 1962, v62, 338.

Received November 18, 1963. P.S.E.B.M., 1964, v115.

Transplant Immunity to Polyoma Virus Induced Tumors. I. Correlations with Biological Properties of Virus Strains.* (29043)

J. DONALD HARE† (Introduced by Herbert R. Morgan)
Louis A. Wehle Virus Research Laboratory, Department of Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The neoplastic conversion of hamster cells by polyoma virus (PV), *in vivo* and *in vitro,* is accompanied by the appearance of an "antigen" specific for tumor cells induced by PV(1,2) but distinct from those induced by

* Supported by grants from Nat. Cancer Inst., U.S.P.H.S. and United Funds of Clayton and Alexandria Bay, N. Y.

† Supported by a Career Research Development Award from the U.S.P.H.S.