RESTORATION OF ALDONOLACTONASE ACTIVITY BY SOMATOTROPIN IN HYPOPHYSECTOMIZED RATS[1]

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It was previously shown that hypophysectomy of rats resulted in decline of urinary excretion, body pool and turnover rate of ascorbate, and that the activity of aldonolactonase was depressed (Salomon and Stubbs, 1961a, b). Since somatotropin could partially repair the defect in synthesis of ascorbate (Salomon and Stubbs, 1961a), as judged by increased urinary excretion and more nearly normal biological half-life, it became of interest to study the effect of the hormone upon the activities of enzymes involved in the conversion of glucuronate to ascorbate. Although it had been reported that Chloretone has little or no effect on ascorbate excretion by hypophysectomized rats, our finding was that stimulation persisted at a high level (Salomon and Stubbs, 1961a). This prompted us to investigate the effect of Chloretone in addition to that of somatotropin.

Chloretone, 9 mg/100g of body weight/day, was given by stomach tube in evaporated milk, the last dose 12 hrs prior to sacrifice. Animals, methods and materials were as summarized earlier (Salomon and Stubbs, 1961b), with the exception of the L-gulonolactone oxidase assay. In that case, experiments showed recovery of ascorbate enzymically produced from L-gulono-γ-lactone to be less than complete. Since addition of reduced glutathione protected ascorbate against oxidation to Roe-negative substances, the following procedure was employed. Microsomes suspended in .05 M tris(hydroxymethyl)aminomethane-HCl buffer, pH 7.6, equivalent to 120 mg/liver,

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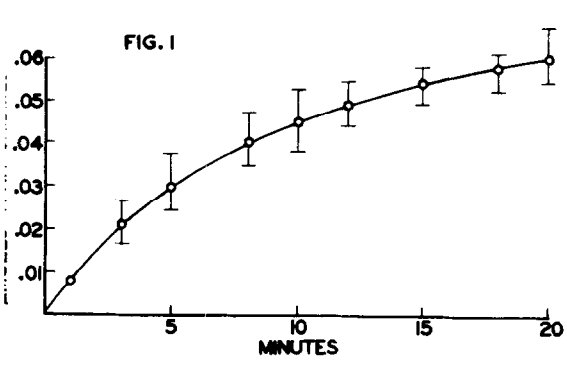
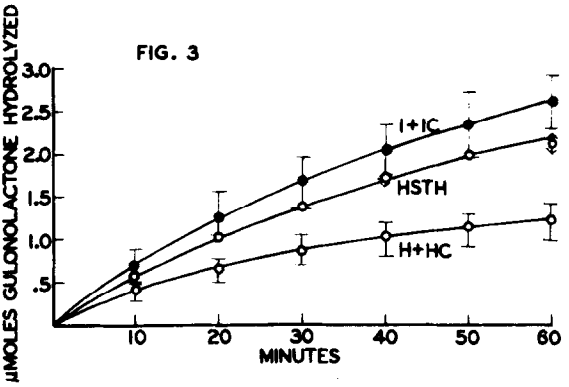
.00042 M L-gulono-γ-lactone, .02 M glutathione adjusted to pH 7.6 with $NaHCO_3$ (all concentrations final), total volume 2.0 ml, were incubated for 1 hr under O_2 at 25°. The reaction was stopped with 1.0 ml 10% trichloroacetic acid, and precipitates removed after centrifugation at 1,000 x g for 15 min. To 1 ml aliquots of supernate was added .25 ml saturated bromine water, an excess as shown by the persistent yellow color, and the excess removed by .1 ml 5% aqueous thiourea. After addition of .6 ml of a solution consisting of 3% 2,4-dinitrophenylhydrazine, .25% thiourea and .1% $CuSO_4 \cdot 5 H_2O$ in 10 N H_2SO_4 (Lowry, Bessey and Burch, 1952, modified), incubation at 37° for 3 hrs, and cooling in an ice bath, 1.5 ml cold 85% H_2SO_4 was added. Then samples were brought to 25°, centrifuged at 1,000 x g for 15 min to remove insoluble precipitates, and read at 540 mμ. Standards and reagent blanks were treated identically; readings followed Beer's law over a wide range of concentrations. Enzyme blanks were identical to test samples excepting omission of substrate. It was found that there was no difference between enzyme blanks whether incubated prior to addition of trichloroacetic acid, or immediately inactivated and set aside for assay without incubation, provided glutathione was present. This method gives close to 70% conversion of gulonolactone. Efficient conversions have also been reported recently by Bublitz (1961).

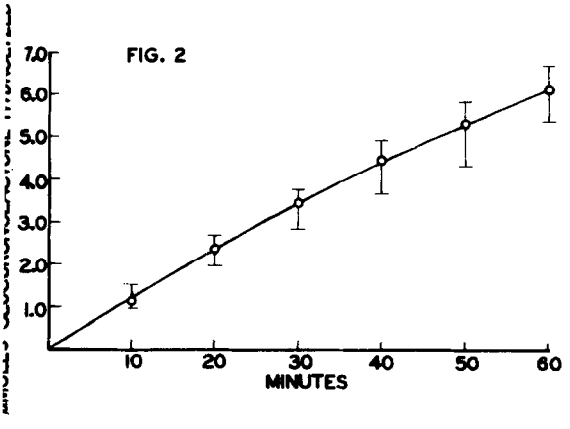
Using the modified method, we were unable to detect any effect of somatotropin* upon the activity of L-gulonolactone oxidase in hypophysectomized rats, nor could we confirm the relatively small decrease in activity following hypophysectomy observed previously (Salomon and Stubbs, 1961b). Similarly, we could not confirm the report by Conney et al. (1961) that the activity of this enzyme was depressed by 50% following chloretonization of intact rats. We believe that the modified assays are reliable because they afforded complete recovery of ascorbate, and that other, perhaps unrelated factors leading to oxidation of ascorbate may sometimes give spurious, low values when suitable protective substances are omitted from the media.

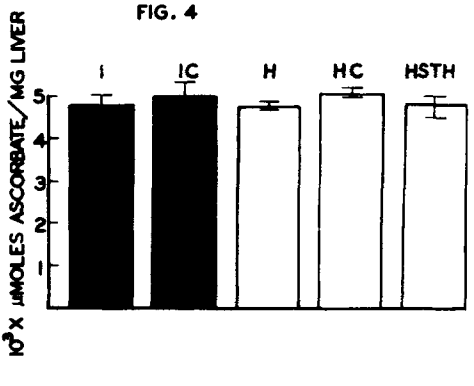
* Somar, The Armour Laboratories, Kankakee, Ill., administered intraperitoneally in doses of 300 μg/day for 14 days.

All results are summarized in Figs. 1-4. TPN-L-hexonate dehydrogenase assays (Fig. 1), uronolactonase assays (Fig. 2) and L-gulonolactone oxidase assays (Fig. 4) on intact (I), intact chloretonized (IC), hypophysectomized (H), hypophysectomized chloretonized (HC) and hypophysectomized somatotropin-treated (HSTH) groups of rats† failed to disclose significant differences under the various experimental conditions, so that the data were treated as replicates in Figs. 1 and 2. Ranges of experimental data are shown, where possible, in all graphs. Fig. 1 is corrected for non-enzymic oxidation of TPNH. A single enzymic activity was affected by somatotropin, and this, aldonolactonase, was precisely that which decreased following hypophysectomy, again to the exclusion of the other enzymes under investigation.









† The number of separate assays in each category, in the order here mentioned, was for Fig. 1: 2, 2, 2, 2, 2; for Fig. 2: 4, 2, 2, 2, 3; for Fig. 3 and 4: 3, 2, 3, 2, 3.

The specificity of the damage of hypophysectomy in this area of metabolism has been pointed out earlier (Salomon and Stubbs, 1961b). To this may now be added the strikingly similar specificity of somatotropin in restoring the activity of aldonolactonase, to about 85% of that of intact rats under these conditions. The parallel changes in ascorbate biogenesis suggest that aldonolactonase activity plays an important, possibly rate-limiting role in this process.

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