THE DEFECT IN ASCORBATE SYNTHESIS BY HYPOPHYSECTOMIZED RATS

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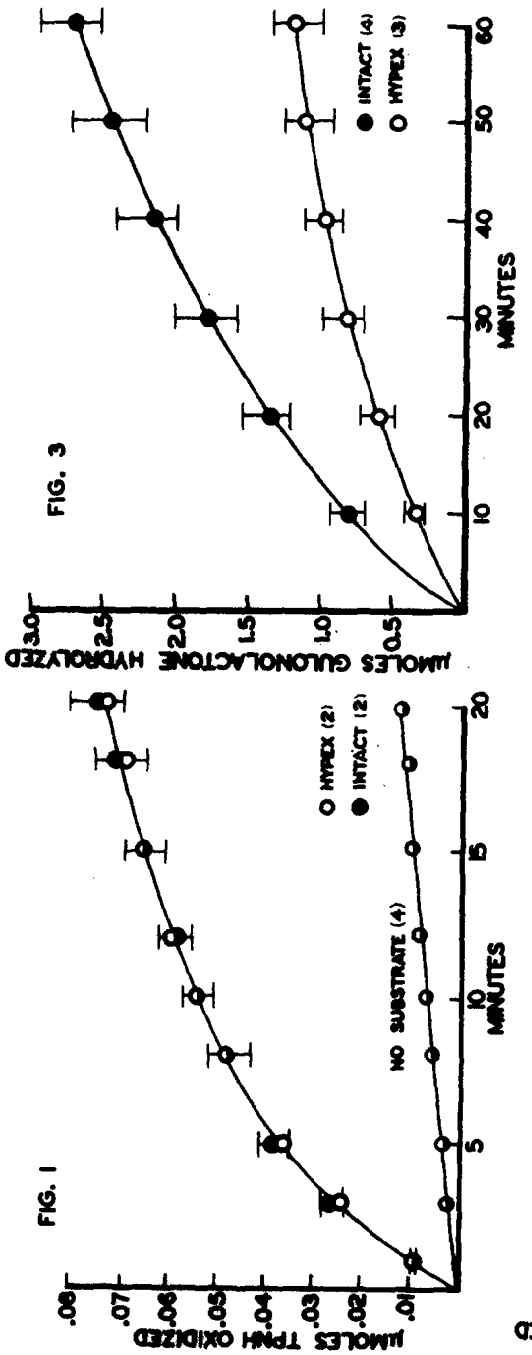
Known enzyme systems, discussed in summary elsewhere (Burns and Conney, 1960), appear to permit synthesis of L-ascorbate from D-glucuronate by the rat through two routes. Reduction by TPNH and TPN-L-hexonate dehydrogenase yields L-gulonate, lactonization by aldonolactonase and oxidation by L-gulonolactone oxidase finally produces L-ascorbate. Alternately, D-glucuronate may be lactonized first by uronolactonase, followed by reduction to L-gulonolactone and oxidation to L-ascorbate. As shown in an earlier report, hypophysectomy of rats induced a metabolic defect in the synthesis of L-ascorbate (measured by turnover, urinary excretion, and tissue analyses) with little and only transient effect on D-glucuronate (measured by urinary excretion) (Salomon and Stubbs, 1961). The data pointed toward a defect following D-glucuronate on the pathway to L-ascorbate, over and above such impairment in turnover of D-glucuronate as might have occurred. Confirmatory evidence for this conclusion is presented below.

Male Sprague-Dawley rats, about 110 grams in weight, were used 8 weeks or more after the operation in the case of hypophysectomized rats (except as indicated) during which weight, sexual maturation and fur texture were observed to ensure absence of hypophyseal function. Animals were decapitated, exsanguinated, and livers perfused with isotonic sucrose. Assay conditions, substantially as described by others (ul Hassan and Lehninger, 1956; Winkelman and Lehninger, 1958; Isherwood, Mapson and Chen, 1960) were as follows. Rat liver was homogenized in 9 volumes of isotonic sucrose, or 4 volumes for the complete system, centrifuged at 10,000 × g for 20 minutes to obtain liver extract free of heavy particles as the supernate, the latter centrifuged at 100,000 × g for 1 hour to yield microsomes and soluble fraction. Uronolactonase assay: Microsomes equivalent to 8.75 mg. liver, .04 M

$NaHCO_3$, .04 M D-glucuronolactone, final volume 2 ml., pH 7.6, 25°, 95% N_2 - 5% CO_2. Measured manometrically by CO_2 evolution for 1 hour. Aldonolactonase assay: Soluble fraction equivalent to 3.75 mg. liver, .04 M $NaHCO_3$, .07 M L-gulono-γ-lactone, final volume 1 ml., pH 7.6, 25°, 95% N_2 - 5% CO_2. Measured manometrically by CO_2 evolution for 1 hour. TPN-L-hexonate dehydrogenase assay: Soluble fraction equivalent to 20 mg. liver, .003 M D-glucuronolactone, .01 M nicotinamide, .001 M TPNH, .01 M KCl, .05 M tris(hydroxymethyl)aminomethane-HCl buffer, final volume 3 ml., pH 7.6, 23°. Measured at 340 mμ for 20 minutes. L-Gulonolactone oxidase assay: Microsomes equivalent to 17.5 mg. liver, .04 M $NaHCO_3$, .06 M L-gulono-γ-lactone, final volume 2 ml., pH 7.5, 25°, O_2. After 1 hour, 1 ml. of 10% trichloroacetic acid added to stop the reaction. Measured by ascorbate production (Lowry, Bessey and Burch, 1952). Assay of complete system: Liver extract equivalent to 120 mg. liver, .05 M tris(hydroxymethyl)aminomethane-HCl buffer, .002 M sodium D-glucuronate adjusted to pH 7.6, .0008 M TPNH, .01 M nicotinamide, .02 M glutathione adjusted to pH 7.6, final volume 2 ml., pH 7.6, 37°, O_2. Reaction stopped by addition of 1 ml. 10% trichloroacetic acid after 1 hour. Measured by ascorbate production (Lowry, Bessey and Burch, 1952). Concentrations cited represent final values. Numbers in Figures 1 - 4 in parentheses indicate number of replicate experiments. Ranges of results are shown, except in Fig. 1, where oxidation of TPNH without substrate showed reproducibility better than indicated by the experimental points. Blanks were used to correct for nonenzymic hydrolysis of lactones, and to correct for the presence of chromogenic substance other than L-ascorbate.

Measurements of activities of enzymes catalyzing the conversion of D-glucuronate to L-ascorbate in preparations from livers of intact and hypophysectomized rats disclosed that uronolactonase and TPN-L-hexonate dehydrogenase were not affected by the operation (Fig. 1 and 2). Indeed, the activity of uronolactonase exhibited a small but consistent increase. TPNH, required for reduction by the TPN-linked dehydrogenase, was previously shown to be not limiting, since its concentration was markedly increased after hypophysectomy (Salomon and Stubbs,



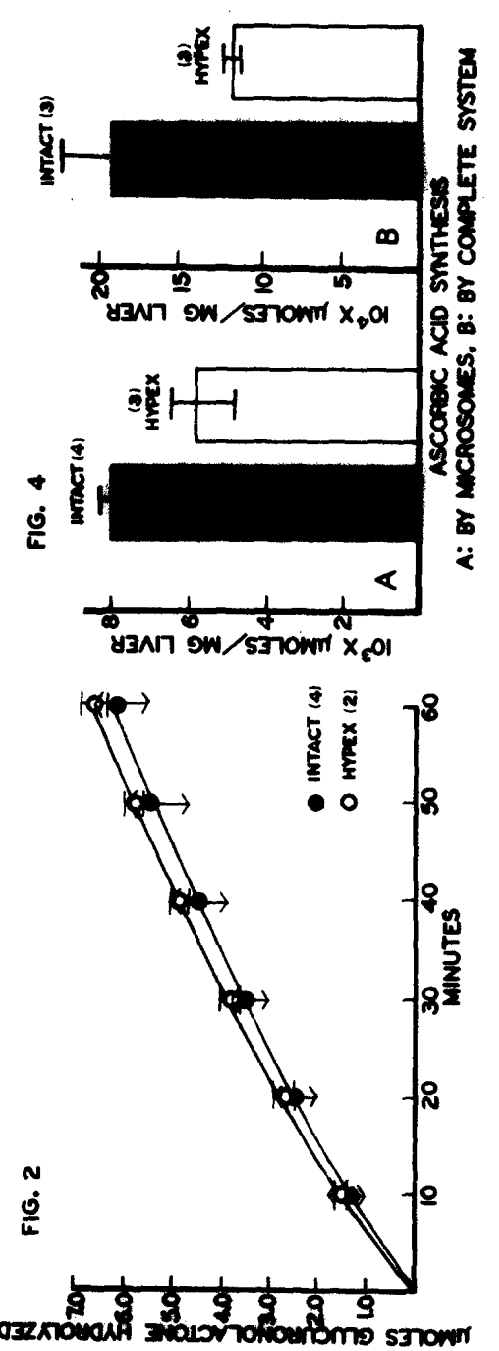


Fig. 1. TPN-L-hexonate dehydrogenase. Fig. 2. Uronolactonase.

Fig. 3. Aldonolactonase. Fig. 4A. L-Gulonolactone oxidase. Fig. 4B. Complete system.

1961). That hypophysectomy induced an impairment of aldonolactonase primarily (56%) and of L-gulonolactone oxidase secondarily (27%) is seen in Fig. 3 and 4A.

In vivo, synthesis was diminished by 50% following hypophysectomy (Salomon and Stubbs, 1961). This may have been the consequence of a joint effect of decreased aldonolactonase and oxidase activities, or of aldonolactonase alone. However, the activity of L-gulonolactone oxidase was not affected sufficiently to implicate it as the solely responsible enzyme. Synthesis of L-ascorbate by liver extracts of hypophysectomized rats utilizing sodium D-glucuronate as substrate (Fig. 4B) was less drastically diminished than in vivo, but also exceeded the degree of diminution of L-gulonolactone oxidase activity. This complete system demonstrated impairment as great 1 week after hypophysectomy (40 and 36% decrease) as after 3 months (34% decrease) indicating, as did earlier data (Salomon and Stubbs, 1961), that the defect appeared promptly and remained permanently. Because no significant differences attributable to changes with time after the operation were noted, the results were treated as replicates in Fig. 4B.

These findings tend to confirm earlier conclusions regarding the occurrence and site of a major defect in synthesis of L-ascorbate by hypophysectomized rats. The disparity of effects of hypophysectomy on enzymic activities in this narrowly circumscribed area of metabolism is of interest.

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