transaminase, this detergent was made use of in an attempt to detach cerebral mitochondrial aspartate transaminase from its structural matrix. Table II (Expt. 2, D) illustrates the activating effect of 1.42% sodium deoxycholate on the enzyme. Unlike liver mitochondrial aspartate transaminase, all of the activated, particulate enzyme activity could be recovered in the soluble supernatant fluid after centrifugation of the deoxycholate-treated suspensions at 140 000 × *g* for 30 min (Expt. 2, E).

The discrepancies in enzyme recovery (Table II, Expt. 2, B + C/A) have not been resolved to date. It should be noted, however, that good enzyme recoveries have been obtained in preliminary studies on the intracellular localization of aspartate transaminase in rat cerebral cortex, in which the enzyme activity of all subcellular fractions was determined in the presence of 1.42% deoxycholate.

Our results on the latency of cerebral mitochondrial aspartate transaminase are in general agreement with the observations of MAY *et al.*[10], as well as with the results of MCARDLE *et al.*[11]. At this time, therefore, the preferred interpretation of the effect of the detergents is that of a lytic action upon those structural mitochondrial lipoproteins which impede optimal interactions between enzyme and substrate in the intact mitochondrion.

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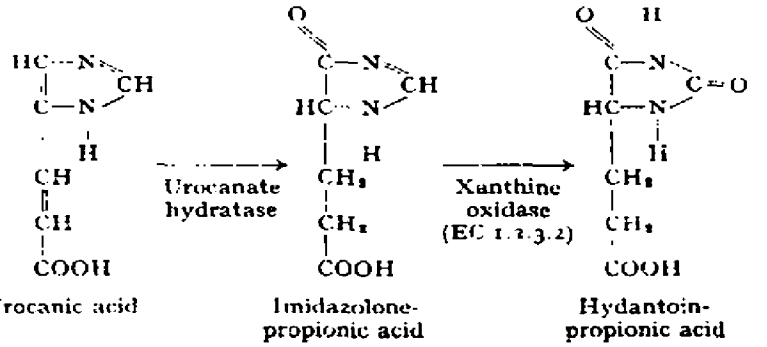
The oxidation of 4(5)-imidazolone-5(4)-propionic acid to hydantoin-5-propionic acid by xanthine oxidase

Administration of radioactive L-histidine to the monkey, human and rat leads to the excretion of L-[^{14}C]hydantoin-5-propionic acid in the urine. From 4–8% of the administered radioactive is excreted in the urine during the first 12 h after in-

jection, with 3% of this excreted dose being hydantoinpropionic acid in the case of the female monkey[1]. Upon injection of hydantoinpropionic acid into both the guinea-pig and rat, there was complete recovery of the unchanged acid from a 12-h urine sample[2]. Using a soluble enzyme fraction from guinea-pig liver the latter authors also established that the hydantoinpropionic acid arises from histidine via urocanic and imidazolone propionic acids.



We have recently found that a commercial preparation of xanthine oxidase, obtained from milk, (Worthington Biochemical Corporation) will readily catalyze the oxidation of imidazolonepropionic acid to hydantoinpropionic acid. Purified beef-liver urocanate hydratase, free of any contaminating imidazolonepropionic acid hydrolase, was routinely used to synthesize imidazolonepropionic acid *in situ* from [2-^{14}C]urocanic acid (ring); the latter was prepared by the action of bacterial histidine ammonia-lyase (EC 4.3.1.3) upon appropriately labeled histidine. To follow the action of xanthine oxidase on imidazolonepropionic acid, the enzyme was incubated in a total volume of 0.2 ml with urocanate hydratase[3] and urocanic acid. Each reaction was stopped by heating at 100° for 60 sec and the immediate addition of 0.05 ml of 2 N HCl. 0.05 ml of each supernatant solution was chromatographed on paper ascendingly in the organic phase of isobutanol–formic acid–water (19 : 2 : 6), the radioactive areas were located by radioautography and the activity of each compound assayed by direct counting of the excised spot.

Fig. 1 shows the time curve for the production of hydantoinpropionic acid from urocanic acid by the action of the two enzymes. Formylisoglutamine, which is not metabolized by the system, reflects to a certain extent the concentration of imidazolonepropionic acid. The latter decomposes spontaneously to formylisoglutamine and 4-oxoglutaramic acid, slowly under the incubation conditions but completely on stopping the reaction[3]. Since the formation of 4-oxoglutaramate leads to the elimination of the labeled carbon atom as [^{14}C]formic acid only the formylisoglutamine, of the two non-enzymic products, appears on the radioautogram. The broken line in Fig. 1 indicates the amount of radioactivity lost as formic acid during the analysis of the reaction products. Fig. 2 shows the dependence of hydantoinpropionic acid production on the concentration of xanthine oxidase when the concentrations of urocanate and urocanate hydratase are maintained constant. Table I indicates the effect of various omissions from the system and illustrates clearly that xanthine oxidase has no effect directly on urocanic acid. Where applicable, heat denaturation of the enzyme was accomplished by incubation at 100° for 10 min.

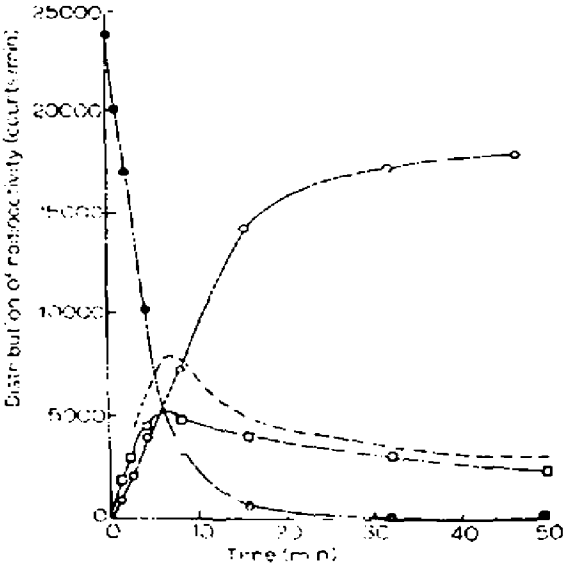


Fig. 1. The production of hydantoinpropionic acid from urocanic acid. Each reaction mixture consisted of 0.02 μmole (0.18 μC) of [2-¹⁴C]urocanic acid, 13 units of beef-liver urocanate hydratase and 24 units of xanthine oxidase in 0.01 M phosphate buffer (pH 7.2). The units of urocanate hydratase activity were those defined by RAO AND GREENBERG[6]. A unit of xanthine oxidase activity was taken as an increase in absorbancy of 0.01/min. at 290 mμ in the system described by AVIS, BERGEL AND BRAY[7]. Urocanic acid, ●—●; *N*-formylisoglutamine, □—□; hydantoinpropionic acid, ○ --○.

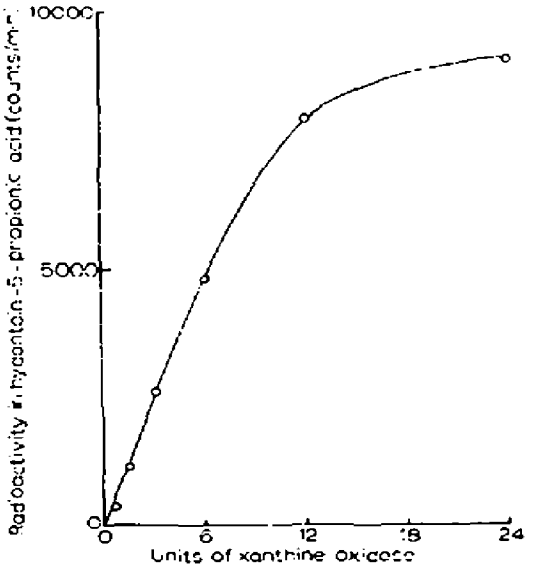


Fig. 2. The dependence of hydantoinpropionic acid production upon the concentration of xanthine oxidase. The reaction conditions were as given in the legend to Fig. 1 but with the xanthine oxidase concentration varied as shown and a reaction time in each case of 10 min.

The oxidation of imidazolonepropionic acid by xanthine oxidase has also been followed spectrophotometrically at 260 mμ using imidazolonepropionic acid isolated as described by BROWN AND KIES[8]. From these studies the rate of oxidation was calculated to be approx. $^1/_{50}$ that of xanthine under similar conditions. Using guinea-pig-liver homogenates, we have now obtained a soluble enzyme fraction whose ability to catalyze the formation of hydantoinpropionic acid has been enriched some

TABLE I

OXIDATION OF IMIDAZOLONEPROPIONIC ACID TO HYDANTOINPROPIONIC ACID BY XANTHINE OXIDASE

The reaction conditions were identical to those given in the legend to Fig. 1 with an incubation time in each case of 30 min. The values (counts/min) given below refer to the distribution of the radioactivity at the end of the experiment.

Experimental conditions	*Urocanate*	*Formylisoglutamine*	*Hydantoinpropionate*
Heat-denatured urocanate hydratase and heat-denatured xanthine oxidase	22 800	12	32
Heat-denatured urocanate hydratase and active xanthine oxidase	24 200	15	40
Active urocanate hydratase, heat-denatured xanthine oxidase	1 470	7900	345
Active urocanate hydratase and active xanthine oxidase	224	2300	17 600

200-fold. This enzyme preparation shows no detectable xanthine oxidase activity. Therefore, although xanthine oxidase will readily catalyze imidazolonepropionic acid oxidation as shown, it appears that there is also a specific enzyme present in the liver of certain mammalian species.

We have used the coupled urocanate hydratase–xanthine oxidase system, and subsequent isolation of the product on Dowex-1 acetate for the synthesis of mg quantities of [^{14}C]hydantoinpropionic acid from [^{14}C]urocanic acid. High yields are obtained by this method without dilution of the initial specific activity (9 μC/μmole) of the starting material.

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