

disulphide (Fig. 1). This is somewhat reminiscent of a neutral xanthophyll recovered by Lederer<sup>5</sup> from freshwater perch (*Perca fluviatilis*) in that that compound gave a single maximum at 495 m $\mu$  in petrol ether and 510 m $\mu$  in carbon disulphide.

The opah does not store the crustacean carotenoid, astaxanthin, but may obtain its carotenoid, deriving ultimately from such a source, through its heavy diet of argonauts.

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### Intracellular Distribution of Xanthine Oxidase in the Rat Liver

THE separation of cytoplasmic particles into fractions by differential centrifugation has been extensively used for the study of the distribution of intracellular cell constituents. Some enzymes of oxidative metabolism have been shown to be localized chiefly in the fraction of the liver containing large particles (mitochondria)<sup>1</sup>. During the course of an investigation of the xanthine oxidase of the rat liver, we have studied the intracellular distribution of this enzyme, since there seem to be no data in the literature.

The xanthine oxidase activity was measured by the oxygen uptake in a Warburg respirometer using a pyrophosphate buffer pH 8.6, as previously described<sup>2</sup>. Xanthine dehydrogenase activity was determined in Thunberg vacuum tubes, and the reduction of the dye triphenyltetrazolium chloride measured colorimetrically as formazan<sup>3</sup>.

Male Wistar white rats were decapitated and livers were rapidly excised, placed in cold 0.25 M sucrose, cut into small pieces and the connective tissue removed by forcing through a gauze. The liver pulp was weighed and homogenized in 9 volumes of ice-cold sucrose. The differential centrifugation was performed according to the procedure of Hogeboom, Schneider and Pallade<sup>4</sup>. All the glassware and the fractions were kept in ice during the operations. The homogenates were centrifuged at 600g for 10 min. The supernatant and the washings were centrifuged at 8,500g for the separation of the mitochondria. The last centrifugation was at 20,000g for 60 min. All the sediments were washed twice with ice-cold 0.25 M sucrose and the washings added to the supernatants. The fractions were checked by staining with Feulgen and Janus green and examined microscopically. Nitrogen was determined in the fractions as a control<sup>5</sup>.

Table 1. INTRACELLULAR DISTRIBUTION OF RAT LIVER XANTHINE OXIDASE

Fractions*	Xanthine oxidase		Xanthine dehydrogenase		Total nitrogen <sup>§</sup>
	Act-ivity <sup>†</sup>	Per-centage	Act-ivity <sup>‡</sup>	Per-centage	
Homogenate	1,077	100	1,270	100	3,295
Nuclei	0	0	0	0	480
Mitochondria	0	0	0	0	769
Submicroscopic particles (microsoma)	0	0	0	0	635
Supernatant (cell sap)	691	64.15	1,325	104.3	1,605

\* Experiments carried out on four rats.

<sup>†</sup> Oxygen (c.mm.) per gm. dry weight/60 min.

<sup>‡</sup> Formazan ( $\mu$ gm.) produced per gm. dry weight/30 min.

<sup>§</sup> Nitrogen ( $\mu$ gm.) per 100 mgm. wet liver.

Table 1 shows the intracellular distribution of xanthine oxidase and dehydrogenase activities in the four fractions studied. The results clearly demonstrate that practically all the enzymic activity is present in the supernatant fluid corresponding to the cell sap containing the soluble proteins of the cell. Little or no activity could be detected for the nuclear, mitochondrial and microsomal fractions. The enzymatic activity of the mixture of the four fractions was 102 per cent of that of the original homogenate. It is interesting to know that uricase was found only in the mitochondria<sup>6</sup>, in contrast to xanthine oxidase, which is absent from this fraction. The decreased percentage obtained for xanthine oxidase in the cell sap as compared with the xanthine dehydrogenase could be explained by the greater stability of the latter as shown by Richert *et al.* in their inhibition studies<sup>7</sup>.

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### Actively Acquired Tolerance to a Mouse Tumour

THE 6C3HED lymphosarcoma<sup>1</sup> is an ascites tumour of mice, apparently of uniform antigenic properties, containing a near-diploid mode of chromosomes<sup>2</sup>. It is invariably lethal to C3H mice and to F<sub>1</sub> hybrids between the original strain and A or DBA mice. The tumour consistently regresses in mice of other strains. Since Billingham, Brent and Medawar<sup>3</sup> have recently demonstrated that mice can be rendered susceptible to skin homografts by the injection of embryos *in utero* with donor-strain tissues, the same method has been applied to the abrogation of resistance of a strain of Swiss mice to the 6C3HED tumour.

Embryos of the ICR (Institute for Cancer Research, Philadelphia, Pa.) strain of Swiss mice were injected *in utero* on the sixteenth to seventeenth day of