Liver Xanthine Oxidase and Q_{O_2} in Relation to Thyroid Activity* (17749)

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The effect of thyroidal activity on liver xanthine oxidase and liver Q_{O_2} was studied for several reasons. Brophy and McEachern(1) reported that the Q_{O_2} of rat liver slices is highly variable in hyperthyroidism, sometimes being abnormally low. Since the rate of oxygen consumption by a rat liver homogenate is dependent upon the xanthine oxidase activity of the liver(2), it seemed possible that the hyperthyroidal depression of the liver Q_{O_2} resulted from a loss of this enzyme. However, the results have shown that the Q_{O_2} of a liver slice, in contrast to an homogenate, is relatively independent of the xanthine oxidase activity of the liver. We have been unable to produce livers with low Q_{O_2} by the administration of thyroxine, but if this can be done in certain rats, it would seem to be unrelated to the liver xanthine oxidase.

The toxic effect of excess thyroxine in limiting the growth rate of young rats on a purified diet is an induced nutritional deficiency that can be prevented by feeding an unidentified factor found in liver(3,4). Normal levels of liver xanthine oxidase are also dependent upon a similar factor from liver(5). The possibility that the administration of thyroxine to rats on a purified diet would alter the liver xanthine oxidase was therefore investigated.

Experimental. Liver Q_{O_2} vs Xanthine Oxidase. The xanthine oxidase activity of each liver homogenate was determined by the method of Axelrod and Elvehjem(2,6). The Q_{O_2} of the liver slice was measured in a similar setup, using oxygen as the gas phase and determining dry weights on alternate slices. Both determinations were made simultaneously on the same liver. Varying levels of liver xanthine oxidase were obtained by feeding adult and immature rats different diets. High normal levels were observed in rats maintained on Purina dog chow, while intermediate and low levels were obtained by feeding young rats a purified 21% casein or 8% casein diet(5) respectively for 1 to 4 weeks. All of the results have been plotted in a scatter diagram, Fig. 1, and show that the Q_{O_2} of the slice was unaffected by the xanthine oxidase activity of the liver unless the latter had been decreased to "zero" levels. The liver Q_{O_2} for 39 rats maintained on chow averaged 6.22 with a standard error of the mean of ± 0.11; the corresponding xanthine oxidase activity was 1377 ± 66 units. When the liver xanthine oxidase was depleted to "zero" levels (actually 50-100 units(2)), the Q_{O_2} of the slice was decreased by 17%, the average for 17 rats being 5.28 ± 0.25 ($P = < 0.01$).

The results show that the level of liver xanthine oxidase is not an important factor in determining the Q_{O_2} of the slice. The metabolic reactions occurring in a slice consume oxygen at a faster and more constant rate than in an homogenate, possibly due to the greater stability of the catalytic nucleotides in the slice. Tipping in xanthine to an actively respiring liver slice of normal xanthine oxidase activity increased the rate of oxygen consumption very little. Glycogen and glucose also did not appear to be substrates for the respiration of a slice, since the Q_{O_2} was unaffected by a 24-hour fast or by

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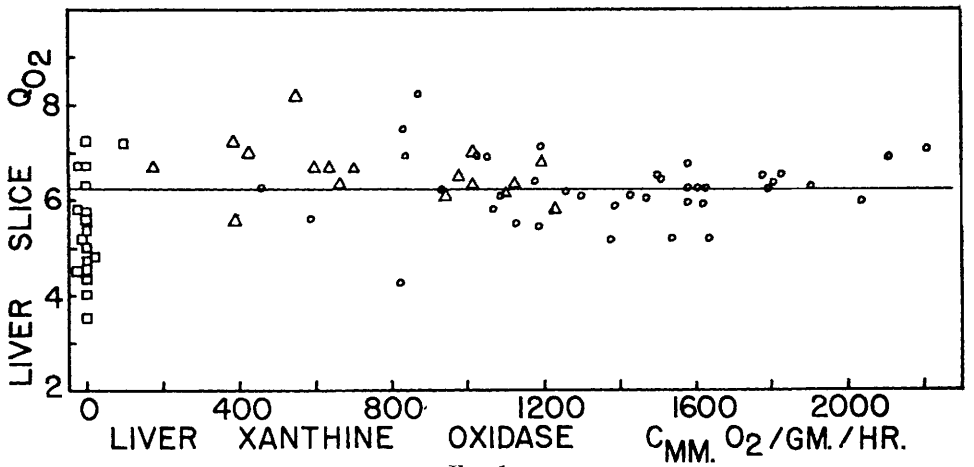

FIG. 1.

Scatter diagram showing the relationship between the Q_{O_2} of a liver slice and the xanthine oxidase activity of the liver. Varying levels of liver xanthine oxidase were obtained by feeding rats different diets: O = chow; △ = purified 21% casein; □ = purified 8% casein.

the addition of 100 mg glucose to the Warburg flask containing slices from either normal or fasted rats.

Thyroxine vs. Liver Xanthine Oxidase and Q_{O_2}. Thyroxine was administered to adult Sprague-Dawley male rats and to young albino female rats maintained on chow post-weaning. From 0.5 to 1.4 mg thyroxine was injected subcutaneously daily, or desiccated thyroid was added to the diet at a level of 0.5% until the rats were sacrificed for analysis of the liver. Rats receiving thyroxine were simultaneously fed chow, the 21% purified casein diet or the 8% casein diet. The low casein diet was fed for 5 days before the thyroxine administration was started while the 21% casein diet was started simultaneously with the thyroxine. 0.3% Thiouracil was also fed in a chow diet.

The results are shown in Table I. The known effect of thyroxine in significantly increasing the liver Q_{O_2} was observed on all 3 diets; in no instance did a low Q_{O_2} result from thyroxine administration. Thiouracil had a smaller but significant effect in decreasing the Q_{O_2}. The previously demonstrated effect of diet on liver xanthine oxidase(5) was again apparent in a comparison of the control groups on each diet. Since the young rats used in these experiments were maintained on chow for 2 to 3 weeks post-weaning, the liver xanthine oxidase was approximately 1500 to 1600 units at the start of the thyroxine studies(5). These levels were maintained in the chow-fed animals, but were decreased to 1100 - 1200 units by the purified 21% casein diet and to almost zero levels by the 8% casein diet. Adult rats showed little effect from the short period on the purified 21% casein diet.

Thyroxine depressed the growth rate approximately 50% in young rats on the purified 21% casein diet, but had no effect on the liver xanthine oxidase. When administered to adult male rats fed chow, thyroxine tended to increase the liver xanthine oxidase. In two additional experiments not recorded in the table, adult male rats fed 1% desiccated thyroid on chow for 11-13 days had average liver xanthine oxidase levels higher than the simultaneous chow fed controls by 48% ($P = <0.01$) and 20% ($P = 0.1$). When administered to rats receiving the low protein diet, thyroxine prevented the marked depletion of liver xanthine oxidase that was observed in the control group. In this respect thyroxine altered the response of liver xanthine oxidase from that characteristic of a low protein diet to an effect characteristic of a starvation regime, inasmuch as this enzyme can be removed almost completely from the liver by a low protein diet but can be decreased only about 50% by starvation(7).

Other Tests. Adult male albino rats main-

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TABLE I.

The Effect of Thyroxine on Liver Xanthine Oxidase and Q_{O_2} When Administered to Rats on Different Diets.

Exp. No.	Sex	Diet	Treatment: Type	Treatment: Duration, days	No. of rats	Body wt, g: Start	Body wt, g: End	Liver X.O.: $CmmO_2/g/hr$ Mean ± S.E.	Liver X.O.: P	Liver Q_{O_2}: Mean ± S.E.	Liver Q_{O_2}: P
1	F	Dog chow	—	—	4	—	145	1873 ± 128	—	6.09 ± .37	—
			.5% dess. thyroid	20	4	114	119	1853 ± 131	1.0	9.44 ± .29	<.01
			.3% thiouracil	22	6	95	120	1587 ± 251	0.3	5.21 ± .20	.07
2	M	Dog chow	—	—	6	—	352	1536 ± 120	—	6.01 ± .12	—
			.5% dess. thyroid	20	2	365	350	2290 ± 30	—	7.72 ± .39	—
			.3% thiouracil	40	6	347	336	1224 ± 98	0.07	5.07 ± .30	.02
3	F	21% casein	—	21	6	93	139	1096 ± 46	—	6.27 ± .14	—
			.5 mg thyroxine	19	6	91	112	1178 ± 55	0.3	9.99 ± .29	<.01
			1.0 mg thyroxine	20	7	94	112	960 ± 23	0.3	9.49 ± .39	<.01
4	F	21% casein	—	15	9	105	133	1241 ± 61	—	—	—
			1.0% dess. thyroid	14	7	108	118	1159 ± 66	0.4	—	—
5	M	21% casein	—	12–32	10	332	379	1468 ± 103	—	6.32 ± .10	—
			1.4 mg thyroxine	9–30	12	320	246	1463 ± 81	1.0	8.75 ± .25	<.01
6	F	8% casein	—	8–13	9	107	110	12 ± 12	—	5.67 ± .39	—
			1.0 mg thyroxine	8–13	8	107	95	752 ± 175	<0.01	9.60 ± .20	<.01

TABLE II.

Effect of Growth Hormone and ACTH on Liver Xanthine Oxidase.

Exp. No.	Diet	Treatment	No. of rats	Body wt, g: Start	Body wt, g: End	Adrenal wt mg/100 g body wt	Liver xanthine oxidase: Mean ± S.E.
1	Dog chow	—	9	222	256	11.3	1866 ± 125
		Growth hormone	9	231	273	13.1	1604 ± 81
		ACTH	9	223	243	14.2	1888 ± 74
2	Horse meat	—	9	227	274	12.8	2162 ± 125
		ACTH	9	224	265	16.5	2176 ± 60
3	Dog chow	—	9	255	262	—	1402 ± 96
		Growth hormone	9	255	284	—	1466 ± 42

tained on chow or horse meat were injected twice daily with Armour's‡ growth hormone or ACTH. A total of 1 mg per rat per day was given for 14 days, after which the rats were sacrificed for a determination of liver xanthine oxidase. The results are shown in the first two experiments of Table II. Neither the growth hormone nor the ACTH had any effect on liver xanthine oxidase. The endogenous respiration of the livers from the rats treated with growth hormone or ACTH was the same as that for the controls. Horse meat gave significantly higher levels of activity than chow. The growth hormone experiment was repeated in female albino rats, and the results, Expt. 3 of Table II, again showed no effect. Methylene blue added to the Warburg flasks gave the same average 1.54 increase in xanthine oxidase activity in the rats treated with growth hormone as was obtained in the controls.

‡ We are indebted to Dr. I. M. Bunding of Armour and Co., for the ACTH and growth hormone.

Summary. The Qo_2 of a liver slice, in contrast to an homogenate, was relatively independent of the xanthine oxidase activity of the liver, since the latter could be varied widely by dietary procedures without affecting the former.

Thyroxine increased the liver slice Qo_2 of rats fed stock chow, a purified 21% casein diet, or a purified 8% casein diet; no hyperthyroidal exhaustion of the liver Qo_2 could be produced.

Thyroxine had no effect on liver xanthine oxidase when rats were fed a purified 21% casein diet, but tended to give higher values with chow-fed adult rats. When administered with a low protein diet, thyroxine prevented the marked depletion of liver xanthine oxidase and gave results more characteristic of starvation than of a low protein diet.

Growth hormone and ACTH had no effect on liver xanthine oxidase.

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Effect of Particle Size of Suspensions of Estrogen Crystals on Duration of Estrus. (17750)

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The implantation of pellets of all hormonal steroids has become a common practice both in the laboratory and in the clinic since the demonstration by Deanesly and Parkes(1,2) that dry crystals or compressed pellets of androgens and estrogens when implanted, acted like a reservoir of the steroid and produced a prolonged physiological effect. Between solutions of steroids in water or oil and the pellets weighing several milligrams, lies a region of particle size which has been little investigated in a systematic manner. Richards, Spruth and Russell(3) showed that a single injection of 500 μg (5,000 I. U.) of an aqueous suspension of small crystals of estrone that would pass a 26 gauge needle, resulting in an average duration of estrus 15-23.5 days in four different groups of castrate rats. The same amount of estrone in oil solution resulted in estrus lasting only 2 days. Recently this report has been confirmed by Simond *et al.*(4) but only high dosage levels of 5,000 to 20,000 I.U. were used.

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