17125. The Xanthine Oxidase Activity of Rat Tissues.*

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The scattered distribution of xanthine oxidase in various organs of different species has been summarized by Morgan.[1] In general, it is absent from embryonic tissue, but appears shortly after birth. It is present in the livers of most, but not all, species and is encountered frequently in kidney, spleen, lung, small intestine and occasionally in pancreas. It has not been detected in muscle, thymus, stomach or large intestine of those species studied.

The purpose of the present communication is to consider 1) the relative amounts of xanthine oxidase in various rat tissues, and 2) the changes in the enzyme activity of these tissues as the liver xanthine oxidase is depleted by dietary means.

Methods. Adult rats weighing 200-300 g were used throughout. Normal xanthine oxidase levels were obtained in rats maintained on Purina dog chow. Liver xanthine oxidase was depleted by feeding rats a purified 8% casein diet[2] for 4 to 8 weeks. The xanthine oxidase activity of various organs was determined by the method of Axelrod and Elvehjem.[3] Added xanthine oxidase was prepared from milk by the method of Ball.[4]

Results. Table I shows the average xanthine oxidase activity of the normal rat tissues studied, together with the effect of adding methylene blue or purified xanthine oxidase. The amount of enzyme present in each tissue giving rise to the activity actually found could not be evaluated by a direct comparison of activities. Added xanthine oxidase was recovered nearly quantitatively when added to lung, spleen, or kidney, but was recovered in increased amounts when added to liver; hence a given weight of enzyme was more active in liver than in the other tissues. The enzyme was destroyed in the presence of homogenized small intestine; not only was the added xanthine oxidase not recovered, but in many determinations the activity fell off rapidly. All values for small intestine were therefore minimal, but alterations with diet were comparable because they were all determined in the same way.

The effect of adding 0.15 cc of 0.0113 M methylene blue in the aerobic determination of xanthine oxidase was not the same for all tissues. When added to the isolated milk xanthine oxidase or to liver homogenate, methylene blue increased the activity by an average of 3.0 and 1.6 times respectively.

TABLE I.
Xanthine Oxidase Activity of Normal Rat Tissues.

		Xanthine oxidase activity						
		$Cmm O_2$/g dry wt/hr			$Cmm O_2$/20 min.			
	No. of determinations	Range	Mean ± S.E.	M. blue added	Present in tissue	X.O. added	Total found	X.O. recovered
Lung	7	395–630	479 ± 30	500	12	17	27	15
Spleen	8	390–665	534 ± 35	525	13.5	17	29	15.5
Kidney	7	0–195	135 ± 23	345	3.5	17	18.5	15
Small intestine	8	450–810	628 ± 42	1150	14	17	17	3
Stomach	6	0–85	42 ± 19	120	—	—	—	—
Liver	12	1475–2320	1862 ± 75	2790	53	18.5	83	30

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1 Morgan, E. J., *Biochem. J.*, 1926, **20**, 1282.

2 Westerfeld, W. W., and Richert, Dan A., *Science*, 1949, **109**, 68.

3 Axelrod, A. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1941, **140**, 725.

4 Ball, E. G., *J. Biol. Chem.*, 1939, **128**, 51.

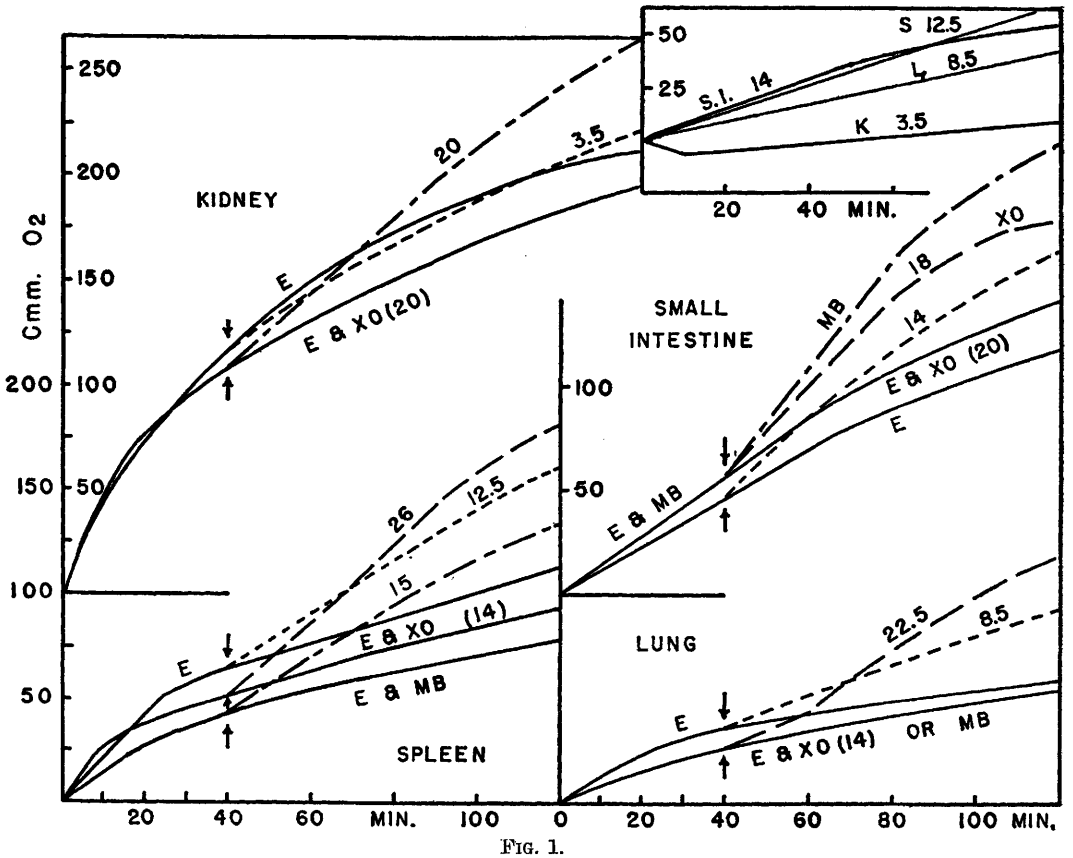

FIG. 1.

Typical oxygen consumption curves for kidney, spleen, small intestine and lung during the determination of xanthine oxidase activity. The effect of adding purified xanthine oxidase or methylene blue to the main body of the Warburg flask is also illustrated.

Solid lines = Endogenous respiration (E) with or without added xanthine oxidase (X.O.) or methylene blue (MB).

Dotted lines = Respiration after tipping in xanthine at the arrows.

Numbers along curves refer to net oxygen uptake in cmm per 20 minutes due to oxidation of xanthine.

Box, upper right: Net oxygen consumption curves due to oxidation of xanthine.

Kidney, small intestine and stomach also gave appreciable increases in xanthine oxidase activity in the presence of methylene blue, and this was very useful in demonstrating the presence of this enzyme in kidney and stomach where the amount was often so small as to be questionable. The xanthine oxidase activity of lung and spleen were unchanged in the presence of methylene blue. The significance of the differences between tissues in respect to this methylene blue effect is not yet apparent.

Brain, skeletal muscle and testes were devoid of xanthine oxidase activity by the usual test as well as in the presence of methylene blue aerobically. Skin contained 100 to 200 units of xanthine oxidase activity ($CmmO_2$/g/hr), and methylene blue increased the activity. Skin xanthine oxidase was not studied in detail because of the difficulty in homogenizing this tissue.

Fig. 1 shows typical oxygen consumption curves for kidney, spleen, lung and small intestine during the determination of xanthine oxidase activity. The effect of adding purified xanthine oxidase or methylene blue to the main body of the Warburg flask is also illustrated. Similar studies with liver will be reported in another communication.

Fig. 2 shows the effect of a purified low-protein diet on the xanthine oxidase activity

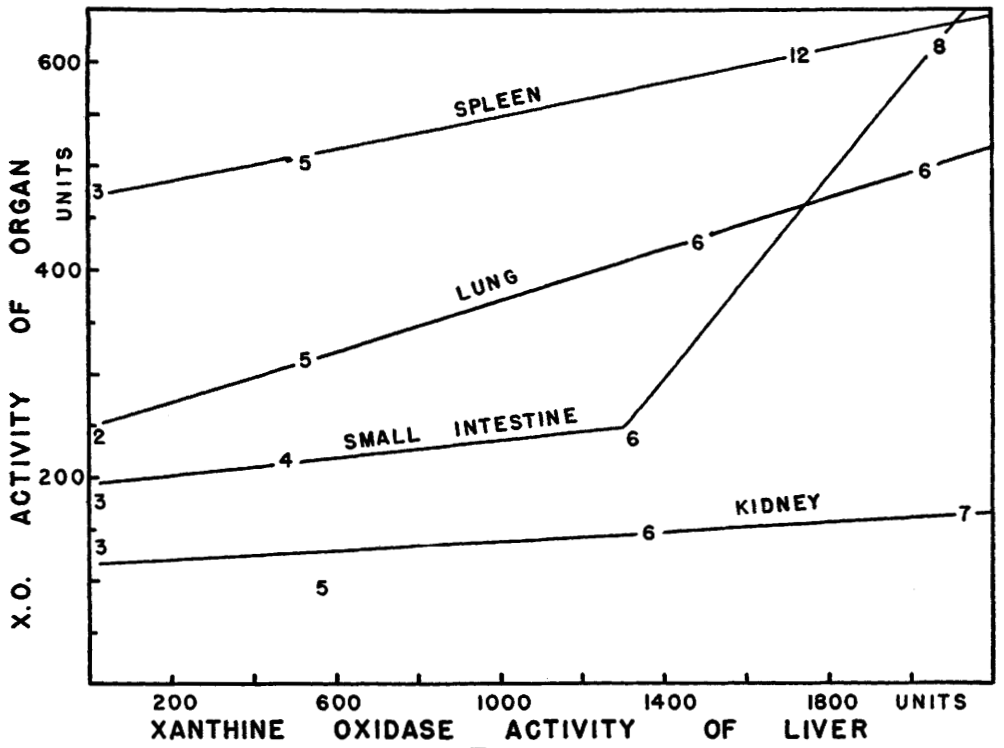

FIG. 2.

Changes in the xanthine oxidase activity of spleen, lung, small intestine and kidney as compared with the changes produced in liver xanthine oxidase by feeding a purified 8% casein diet. Numbers along curves refer to the number of determinations that were averaged to obtain each point on the curve.

of these various tissues in comparison with the changes observed in the liver.

Individual rats varied greatly in length of time on the diet required to deplete the liver of xanthine oxidase. At 4 to 5 weeks, 11 rats had 0, 0, 0, 0, 385, 395, 455, 635, 705, 1160, and 1580 units of liver xanthine oxidase; after 8 weeks, 4 of 5 rats had no liver xanthine oxidase activity. Inanition, which is known to decrease the liver xanthine oxidase,[5] was not a factor in these experiments with adult rats, since body weights increased consistently on the purified low protein diet. Rats were analyzed arbitrarily after 2 to 8 weeks on the diet, and the results were then correlated on the basis of the liver xanthine oxidase found; the data from rats with similar liver xanthine oxidase activities were grouped together without regard to the duration of the individual dietary periods.

[5] Miller, L. L., *J. Biol. Chem.*, 1948, **172**, 113.

As the liver xanthine oxidase activity fell from over 2000 units to zero the small intestine lost about 2/3 of its activity rapidly, but then remained fairly constant at the lower level; lung lost about ½ of its activity in a gradual manner; losses in the spleen and kidney were 20-30%, which were not enough to be statistically different from the normal levels in this limited series. That portion of the enzyme which could be lost readily from the small intestine was depleted more rapidly than the liver. The xanthine oxidase activities of the other tissues were much more refractory to dietary changes than was the liver enzyme.

Assuming that the intact structures maintain the same relative enzyme activities as exhibited in the homogenates, then the total xanthine oxidase activity of a normal mature rat would be distributed approximately as follows: 67% in liver, 20% in skin, 8%

in small intestine, 2% each in spleen and lung, and 1% in kidney. When the liver has been depleted to zero xanthine oxidase activity by a purified low protein diet, the entire rat has lost about 75% of its original enzyme activity (assuming the skin is relatively unchanged).

Summary. The average xanthine oxidase activities in $Cmm O_2$/g dry weight/hour for adult rat tissues were: Liver 1862, small intestine 628, spleen 534, lung 479, kidney 135, stomach 42, skin 100-200, brain 0, muscle 0, testes 0. Two-thirds of the total activity in the entire rat was in the liver.

Methylene blue added to the Warburg flask in the aerobic determination increased the xanthine oxidase activity in liver, kidney, stomach and small intestine, but not in lung or spleen. Added xanthine oxidase was recovered quantitatively in the presence of lung, spleen and kidney; greater than added amounts of activity were recovered from liver and decreased amounts from small intestine.

As the liver xanthine oxidase was depleted by a purified low protein diet, the small intestine lost about 2/3 of its activity, and lung lost about ½. Losses from the spleen and kidney were small. The entire rat lost ¾ or more of its xanthine oxidase activity.

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17126. Relationship Between Protein Osmotic Pressure and Density in Plasma from Cats, Dogs, and Humans.

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During the course of experiments on the effective osmotic pressure of the plasma proteins in the capillary circulation[1] we have had occasion to compare protein osmotic pressure with density in a large number of samples of plasma from cats, dogs, and humans. We were surprised to find that for any given density the protein osmotic pressure of human plasma is higher than that of plasma from cats or dogs. The data given below may prove useful to other investigators who wish to use plasma density as a measure of the osmotic activity of the plasma proteins in these species.

Methods. Blood was drawn from the carotid arteries of cats and dogs anesthetized with Nembutal. Human blood was obtained by venipuncture. Heparin was used to prevent coagulation. Low protein concentrations were obtained by diluting the plasma with Ringer's solution (0.9% NaCl, 0.042% KCl, 0.024% $CaCl_2$, 0.020% $NaHCO_3$) of density 1.0068 relative to water at the same temperature. High protein concentrations were obtained by evaporating the plasma in cellophane bags placed in front of a fan. The bags were then sealed close to the liquid level and the concentrated plasma dialyzed against cold Ringer's solution until ionic equilibrium was established as indicated by the electrical conductivity.

Densities were determined by the Falling Drop Method of Barbour and Hamilton.[2] The standard deviation from the means of 68 duplicate determinations performed with this method was ± 0.00013 g/cc. Protein osmotic pressure measurements were made across collodion membranes using a Hepp osmometer.[3] All the results were corrected to 37°C by multiplying the observed values by the factor $273 + 37 \div 373 + t$, in which t = room

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[1] Pappenheimer, J. R., and Soto-Rivera, A., *Am. J. Physiol.*, 1948, **152**, 471.

[2] Barbour, H. G., and Hamilton, W. F., *J. Biol. Chem.*, 1926, **69**, 625.

[3] Hepp, O., *Z. f. d. Gesamte Exp. Med.*, 1936, **99**, 709.