of the blood proteins appears to have occurred at 8 hours. Unfortunately, the experiment was so designed that the plasma volume was not measured at that time.

It is known that in congestive heart failure the onset of diuresis is preceded by a fall in the specific gravity of the plasma, presumably due to an increased hydration of the blood plasma(4). The resultant increase in blood volume is thought to be responsible for the diuresis. A similar mechanism may have been in operation in the present study; this would explain the decrease in the thiocyanate space and in the body weight observed at 72 hours.

The changes which were observed in the fluid content of the vascular compartment may have been due to a primary decrease in cardiac output, with a consequent decrease in capillary pressure. However, a decrease in the venomotor tone, an increase in the interstitial fluid pressure, or both, may also cause the changes noted. Whether the results were due entirely to the glycoside's effect on the heart or whether there was also an extracardiac effect is not known. A more detailed study of the problem is now in progress.

Summary. The intravenous administration of 1.2 mg of digitoxin to 5 male subjects with no cardiovascular disorder was found to cause a significant decrease in the serum protein concentration and an increase in the total circulating protein content within 8 hours. The results were interpreted as indicating an increase in the water and protein content of the vascular compartment.

4. Stewart, H. J., *J. Clin. Invest.*, 1941, v20, 1.

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Xanthine Oxidases in Different Species.* (18452)

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It has been assumed tacitly that the xanthine oxidase activities demonstrated in different organs from various species(1) were due to the same enzyme, and that this enzyme was similar or identical with the better-known milk xanthine oxidase. A previous study(2) showed that the enzyme occurring in rat liver was different from milk xanthine oxidase inasmuch as only the former could be inhibited by antabuse.† This antabuse inhibition was overcome by the addition of methylene blue to the aerobic system, or by heating the liver homogenate at 56° for 30 minutes. The latter treatment destroyed some of the original aerobic activity of the enzyme, but its dehydrogenating ability was not affected. This evidence was interpreted(2) as showing that xanthine oxidase occurred in rat liver as a complex of two distinct enzyme activities (dehydrogenase and oxidase) closely associated with some other unidentified constituent that conferred antabuse sensitivity upon the complex.

This report describes the application of the same techniques to other selected tissues in order to determine whether "xanthine oxidase activity" is uniformly due to the same kind of enzyme. Ignoring minor differences for purposes of generalization, the xanthine oxidase of mammalian tissues was similar to the rat liver enzyme. The xanthine oxidase of bird tissues, however, was fundamentally a dehydrogenase, since it was nearly devoid of autooxidizable characteristics. Evidence is

* This work was supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

1. Morgan, E. J., *Biochem. J.*, 1926, v20, 1282.

† Antabuse, tetraethylthiuram disulfide, was supplied by Ayerst, McKenna and Harrison, Ltd.

2. Richert, D. A., Vanderlinde, R., and Westerfeld, W. W., *J. Biol. Chem.*, 1950, v186, 261.

TABLE I. Effect of Antabuse, Methylene Blue, and Heating at 56° on Xanthine Oxidase Activities of Tissues from Different Species.

Tissue studied	No. of exp., avg	Xanthine oxidase activities in cmm O_2/20 min./flask: Original tissue —	Original tissue + M.B.	+ antabuse (0.85 mg/cc) —	+ antabuse (0.85 mg/cc) + M.B.	Homogenate heated at 56° for 30 min. —	Homogenate heated at 56° for 30 min. + M.B.	Homogenate heated at 56° for 30 min. + antabuse
Rat liver*	6	34	60	12	60	15	55	19
" lung	3	8	10	4	9	13	10	11
" spleen	3	12	14	7	16	22	17	21
" kidney	3	2	6	1	7	3	6	2
" intestine†	3	9	24	0	18	11	30	0
	2	36	53	4	41	33	66	3
Mouse liver	3	40	59	8	59	22	59	23
Cat liver	5	12	31	3	30	12	31	7
Guinea pig liver	6	13	25	0	25	12	23	4
Dog liver	5	1	10	2	10	3	10	2
Rabbit liver	3	0	6	0	6	0	2	0
Pig "	2	11	17	1	17	11	14	8
Cow "	3	7	12	1	12	7	12	2
Frog "	2	4	4	0	4	0	3	0
Pigeon kidney‡	2	1	26	0	35	0	40	0
Chicken liver§	6	6	74	5	102	3	110	4
Turkey liver	3	0	64	4	67	1	77	1

* Published previously (2) but included for comparison.

† First group of 3 exp. was determined by tipping in xanthine substrate after 40-min. incubation. In next group of 2 exp. hypoxanthine substrate was added after 10 min. Results are not directly comparable since different rats were used.

‡ Values reported here may be lower than usually encountered, since about twice this activity was found in other incomplete exp.

§ Hypoxanthine substrate was added after 40-min. incubation.

not yet available which would allow a characterization of the "mammalian xanthine oxidase" as a complex of 2 separate enzymes (oxidase and dehydrogenase) so firmly bound together that they cannot be separated by the usual techniques, or whether this enzyme exhibits two activities because it contains 2 prosthetic groups within the same molecule. A study of purified chicken liver xanthine dehydrogenase is now in progress in order to determine the characteristics of the prosthetic group of this enzyme.

Methods. The xanthine oxidase activity of each tissue was determined manometrically by the procedure of Axelrod and Elvehjem (3,4). A weighed sample was homogenized with 2½ volumes of the phosphate buffer to give a concentrated homogenate. Aliquots were further diluted with buffer or buffer plus antabuse and rehomogenized to give a 1 + 5 dilution of the original tissue. The antabuse concentration was 1 mg per cc of homogenate or 0.85 mg per cc of fluid in the Warburg flask. Another aliquot of the concentrated homogenate was heated in a 56° water bath for 30 minutes, cooled, and then treated like the unheated sample. When necessary, tissues from several animals were pooled to make the concentrated homogenate. All Warburg flasks used in determining the xanthine oxidase activity contained 1.7 cc of homogenate (283 mg fresh tissue) in a total fluid volume of 2.0 cc. The effect of methylene blue was determined by making 0.15 cc of 0.0113 M methylene blue a part of the total 2 cc volume. The substrate, 0.15 cc of 0.05 M xanthine, was tipped into one of a pair of flasks after a preliminary 40-minute incubation period that partially exhausted the endogenous respiration. The activities have been recorded in net oxygen consumption per 20 minutes per flask, and are directly comparable.

Results. The results are shown in Table I.

3. Axelrod, A. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1941, v140, 725.

4. Richert, D. A., Edwards, S., and Westerfeld, W. W., *J. Biol. Chem.*, 1949, v181, 255.

From 2 to 6 complete experiments on each tissue were averaged to give the recorded values. In addition to the general conclusions recorded in the summary, the following points may be noted. Dog liver was previously reported(1) to be free of this enzyme, but its presence could readily be demonstrated by the addition of methylene blue to the aerobic test system. The pigeon was the only species in which the liver was found to be completely free of xanthine oxidase; no activity was detected in any of 6 experiments with xanthine or p-hydroxybenzaldehyde as the substrate in the presence or absence of methylene blue.

The xanthine oxidase of mouse liver was unchanged by a total body x-ray irradiation of 600 r. Control mice had liver xanthine oxidases in the presence and absence of methylene blue of 36 and 20 cmm O_2/20 min. respectively, while the mice analyzed 3 to 6 days after irradiation‡ had corresponding values of 42 and 18.

Human livers obtained at autopsy after accidental or other deaths had little xanthine oxidase. In the usual determination, values of 0 to 5 cmm O_2/20 min. were obtained, and these were increased 2 fold by the addition of methylene blue.

Summary. A study of the xanthine oxidases in selected tissue homogenates from different species showed that the enzymes present in bird tissues was a dehydrogenase rather than an oxidase, since the aerobic activity of the enzyme was negligible in comparison with its activity in the presence of methylene blue. The enzyme present in mammalian tissues was an oxidase whose aerobic activity could generally be increased from 1.5 to 2.5 times by the addition of methylene blue to the aerobic test system. In all cases the aerobic activity of the xanthine oxidase was inhibited from 40 to 100% by antabuse, and this inhibition was completely overcome by the addition of methylene blue. Heating the tissue homogenate at 56° for 30 minutes gave decreased aerobic xanthine oxidase activities with rat and mouse liver but no change with most mammalian tissues; the antabuse inhibition was eliminated to varying degrees with different tissues. The dehydrogenase enzyme or the dehydrogenase portion of the mammalian xanthine oxidase complex was unaffected by antabuse or by the heating procedure, since the activity in the presence of methylene blue remained constant irrespective of what happened to the aerobic activity.

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Reactions of Albino Rats to Injections of Dextran. (18453)

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Dextran, a long-chain polysaccharide, in 6% concentration in physiological saline solution, has been used as a plasma substitute in the treatment of hemorrhagic and burn shock (1-4). In experiments with this substance in the albino rat, consistent reactions have been observed.

Reactions in 29 albino rats, 150-250 g, have been observed in our laboratory when 0.5 ml 6% Dextran per 100 g body weight was injected into the tail vein. All rats showed reactions, lasting for a period of 2 or

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1. Gronwall, A., and Ingelman, B., *Nature,* 1945, v155, 45.

2. Bohmansson, G., Rosenquist, H., Thorsen, G., and Wilander, O., *Acta Chir. Scand.,* 1946, v194, 149.

3. Lundy, J., *et. al., Proc. Staff Meet. Mayo Clinic,* 1947, v22, 357.

4. Turner, F. P., *et al., Surg. Gynec. and Obst.,* 1949, v88, 661.