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The Species Distribution of Xanthine Oxidase

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1. The distribution of xanthine oxidase in blood and tissues of various animals was studied by means of a radioactive assay capable of detecting 10^{-7} unit of enzyme. The method was shown to be applicable to tissues with a high uricase content. 2. Of 16 mammalian species examined, six had low concentrations of xanthine oxidase in the serum. In six non-mammalian species, no activity was detected in the serum. 3. The enzyme was not found in the blood cells of any mammals, but was present in the nucleated red blood corpuscles of chicken, turtle and tortoise. 4. Studies of the tissue distribution in four species demonstrated high activities in the liver and intestinal mucosa and consistently low activities in skeletal muscle, heart and brain. 5. There is a rough correlation between the activity of enzyme in serum and its activity in lung tissue in 12 mammalian species. In the dog, left-atrial blood had higher concentrations of xanthine oxidase than right-atrial blood.

A radioactive assay of xanthine-oxidase activity has been developed by al-Khalidi, Nasrallah, Khachadurian & Shammaa (1965). This method allows the determination of 10^{-7} international unit [King & Campbell (1961): see also *Report of the Commission on Enzymes of the International Union of Biochemistry*, chapter 2 (1961) (New York: Pergamon Press)]. Serum from normal humans revealed activity below 0·5 milliunit/l., whereas in patients suffering from acute liver disease serum concentrations of several hundred milliunits/l. were detected. This indicates the usefulness of this test in the diagnosis of liver injury (Shammaa, Nasrallah, Chaglassian, Khachadurian & al-Khalidi, 1965).

In experiments designed to test the effect of liver damage in animals on serum concentrations of xanthine oxidase, it was found that the normal dog has high concentrations of serum xanthine oxidase. A survey of xanthine-oxidase concentrations in the blood of various animals was therefore undertaken with the aim of finding an experimental animal that, like man, had a low concentration of xanthine oxidase in serum. A wide range of variation in the serum concentration was observed among 22 species.

In an attempt to explain these variations, the distribution of enzyme in the tissue was studied in certain species. No survey of xanthine oxidase in blood and only one survey of xanthine-oxidase activity in a variety of animal tissues has so far been published (Morgan, 1926), and these results are only semi-quantitative. Our method is far more sensitive than that used by Morgan (1926) and our results include data for several species not previously investigated.

MATERIALS AND METHODS

Sources of animals and collection of blood. Laboratory animals such as rat, mouse, guinea pig, rabbit, cat and dog were supplied from the University animal house. The other animals were collected from farms or markets.

Blood from small animals was obtained by cardiac puncture and from larger animals by venepuncture. The fox and badger were captive animals bled by cardiac puncture.

Xanthine-oxidase activity was measured in the serum and in some cases also in heparin-treated blood.

[8-^{14}C]Xanthine was prepared from [8-^{14}C]guanine (The Radiochemical Centre, Amersham, Bucks.) by deamination with nitrous acid and was purified on a Dowex 50 (H^+ form) column as described by al-Khalidi *et al.* (1965).

Treatment of tissues. Tissues from various organs of the cat and the dog were taken under anaesthesia. Tissues from sheep and cow were obtained fresh from the slaughterhouse. All tissues were kept on ice until they were homogenized, which was usually 30–60 min. after removal. The tissues were homogenized with 9 vol. of 0·05 M-potassium phosphate buffer, pH 7·4, in a micro-blender (Omnimixer; Ivan Sorvall Inc., Norwalk, Conn., U.S.A.). The whole homogenate and dilutions thereof were assayed for xanthine-oxidase activity.

Determination of the enzyme. Xanthine-oxidase activity was measured as described by al-Khalidi *et al.* (1965). A 1 ml. sample of blood, serum or tissue homogenate appropriately diluted to contain not more than 50 microunits of the enzyme is incubated for 3 hr. at 37° with 2·5 ml. of 0·05 M-phosphate buffer containing 0·1 m-mole of [8-^{14}C]-xanthine (approx. 10^5 counts/min.). The reaction is stopped, and protein precipitated, by the addition of 1 ml. of 20%

(w/v) trichloroacetic acid. After centrifugation, the protein-free filtrate is placed in a boiling-water bath for 20 min. to hydrolyse any nucleotide formed. Then 2 ml. of the filtrate is transferred to a Dowex 50W (H^+ form; 200–400 mesh) column (30 cm. × 0·2 cm.^{2}) and the uric acid quantitatively eluted with 8 ml. of 0·1N-HCl. A 1 ml. sample of the 10 ml. eluate is plated and counted. The assay is standardized as follows. Cream xanthine oxidase, prepared by the method of Ball (1939) and supplied by L. Light and Co. Ltd., Colnbrook, Bucks., is appropriately diluted in 1% crystalline bovine serum albumin and assayed by the method of Kalkar (1947) and the concentration of the enzyme in international units is calculated. A sample is then further diluted in 1% albumin to contain about 20 microunits/ml. and assayed by our procedure. A factor is calculated by which the number of counts/min./ml. of the column eluate is divided to give milliunits of enzyme/l. of serum or extract. A unit of enzyme is defined as the amount of enzyme which catalyses the aerobic oxidation of 1 μmole of xanthine/min. at 25° and at saturating concentrations of xanthine.

Reliability of the assay in the presence of uricase. [^{14}C]-Uric acid was prepared by incubating [8-^{14}C]xanthine with cream xanthine oxidase and isolating the uric acid on a Dowex 50 column as described above. The HCl was evaporated to dryness *in vacuo* and the uric acid dissolved in 0·05M-phosphate buffer, pH 7·4. Known amounts of the radioactive uric acid were incubated under the standard condition of the assay with homogenates of muscle, brain, liver and kidney from a cat. No loss of radioactivity occurred in the uric acid fraction. Thus the products of uricase action on uric acid appear in the uric acid fraction in our column assay and the assay is therefore applicable to the determination of xanthine oxidase in tissues even if they contained high concentrations of uricase.

RESULTS

Table 1 shows average values for the blood and serum xanthine-oxidase activities in the various species. Variations among individual animals of the same species were usually below 30%, the widest variation being about twofold.

Among the mammals examined the sheep, pig, cat, camel and goat had low activities of serum enzyme comparable with those observed in man. By contrast the sera of cow, dog, donkey, badger, guinea pig, rabbit, rat and mouse were rich in the enzyme. In the four avian species tested, there was no appreciable activity in the serum, but the chicken exhibited enzyme activity in the whole blood. Similarly, in the turtle and the tortoise the enzyme was not found in the serum but found in the whole blood. Estimation of the enzyme in chicken blood, red blood corpuscles free of platelets, and white blood corpuscles, showed that most of the enzyme in blood could be accounted for by the enzyme found in the red cells.

Table 2 shows that in the four animal species examined the enzyme was abundant in the liver, whereas it was scarce in heart, muscle and brain.

Table 1. *Blood xanthine oxidase*

Experimental details are given in the text.

Species	No. of animals tested	Xanthine oxidase (milliunits/l.) Blood	Serum
Man (*Homo sapiens*)	40	0	0
Cat (*Felis domesticus*)	3	0·3	0·6
Dog (*Canis familiaris*)	3	23	44
Fox (*Vulpes vulpes*)	2	—	12
Badger (*Meles meles*)	3	—	207
Goat (*Capra hircus*)	2	0	1
Cow (*Bos taurus*)	5	62	142
Sheep (*Ovis aries*)	3	0	0
Camel (*Camelus dromedarius*)	2	—	0·7
Pig (*Sus domesticus*)	2	0	0
Horse (*Equus caballus*)	2	—	40
Donkey (*Equus asinus*)	3	—	29
Rabbit (*Lepus cuniculus*)	3	14	24
Guinea pig (*Cavia cobaya*)	2	63	125
Rat (*Mus rattus*)	3	52	101
Mouse (*Mus musculus*)	2	—	423
Duck (*Anas platyrhyncos*)	2	0	0·9
Goose (*Anser anser*)	2	0	0·8
Pigeon (*Columbia livia*)	2	0	0·9
Chicken (*Gallus domesticus*)	2	16	1·1
Tortoise (*Testudo graeca*)	2	15	0·3
Turtle (*Eretmochelys imbricata*)	2	8·8	0

Table 2. *Tissue distribution of xanthine oxidase*

Experimental details are given in the text. The values in the Table represent the average of the two individual results; these usually agreed within 30%.

Tissue	Xanthine oxidase (milliunits/g. wet wt. of tissue) Cat	Dog	Sheep	Cow
Liver	17	19	12	29
Lung	6·0	24	0·032	48
Kidney	4·8	1·8	0·0035	1·6
Skin	0·49	1·5	1·0	5·7
Heart	0·01	0·3	0·007	0·22
Muscle	0	0·2	0·022	0·33
Pancreas	1·5	0·3	0·015	0·006
Intestine (whole)	—	19	0·058	12
Intestinal mucosa	19	24	—	—
Colon	1·0	2·1	0·005	6·1
Thyroid	0·05	2·5	—	—
Adrenal	0·06	0·71	0·015	—
Uterus	0·73	0·8	—	—
Diaphragm	0·04	0·7	—	—
Ovary	0·30	0·6	—	—
Spleen	0·036	2·1	0·22	20
Bone marrow	0·12	0·75	—	—
Adipose	0·06	1·2	0·004	0·20
Mesentery	0·05	1·4	0·008	—
Brain	0	0·07	0·005	—

Table 3. *Release of xanthine oxidase from lung*

Experimental details are given in the text. In each case the values are means ± S.D. of ten determinations.

Dog	Serum xanthine oxidase (milliunits/l.) From right ventricle	From left ventricle	*P*
1	26·4±3·6	32·8±5·1	0·002
2	8·4±2·1	11·2±1·9	0·002

In the cow and the dog there was a wide distribution of the enzyme with especially high values in the lung. In the cow the spleen and kidney also contained appreciable amounts of the enzyme. Xanthine-oxidase activities (in milliunits/g.) in lung tissue from the following species with low serum xanthine-oxidase concentrations were: man, 0·014; sheep, 0·030; camel, 0·015; goat, 0·076; cat, 6·0. The activities of the enzyme in lung tissues from species with high serum xanthine-oxidase concentrations were: rabbit, 0·46; dog, 24; rat, 25; guinea pig, 5·9; cow, 51; mouse, 15.

To test the possibility that xanthine oxidase leaks from the lungs of animals with high lung concentrations of xanthine oxidase, simultaneous samples of blood were obtained from the right and left sides of hearts of two dogs under anaesthesia. Each sample was assayed in ten replicates. The results (Table 3) show a significantly higher concentration in the serum from the left heart than in serum from the right.

DISCUSSION

Our results agree in general with those of Morgan (1926) if we assume that his method of assay could detect 2–5 milliunits of enzyme/g. of tissue. An exception is that he reported the absence of enzyme in dog liver. This could have been due to the rapid autolysis that occurred when he preincubated liver tissue for 24 hr. to decrease the endogenous reduction of methylene blue.

The sensitivity of our method enables us to conclude that the enzyme is virtually absent from the sera of several mammals. The presence of the enzyme in serum or its concentration does not correlate with phylogenetic relationships; closely related animals may have very different serum concentrations.

The tissue distribution of the enzyme in two related pairs of animals (cat and dog, sheep and cow) was studied. Though no clear pattern is seen, the lung tissue of both animals with high serum concentrations of xanthine oxidase (dog and cow) is rich in the enzyme, whereas the lungs of the cat and sheep contain relatively smaller amounts. In the cow the kidneys and the spleen also have high amounts of the enzyme and the serum concentration in this animal is about three times that of the dog. The rough correlation between the concentration of the enzyme in the lungs and the serum suggests that the high serum concentration may be due to leakage of enzyme from the lung. This assumption is supported by the results in Table 3.

Variations were also noted in the distribution of the enzyme within the blood. Washed red cells from man were free of enzyme, and in all mammals that were examined the concentration of enzyme in whole blood was about half the serum concentration, indicating that the blood cells did not contain substantial amounts of enzyme. Though serum from the four birds and the two reptiles (species having nucleated red blood cells) had low enzyme concentrations, whole blood from the two reptiles and from chicken contained substantial amounts of the enzyme.

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