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Catalase from Horse Kidney and Human Liver *

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Catalase was first crystallized from beef liver in 1937, [1] and since that time catalases have been prepared from horse blood and liver, beef blood, and human blood.[2, 3, 4]

It has long been known that the tissues richest in catalase are blood, liver, and kidney; [5, 6] and since the only difference between the catalases of horse blood and liver is the one verdohemochromogen group in liver catalase [4], it seemed of interest to prepare catalase from horse kidney. Human liver catalase was also prepared in order to compare it with the known blood catalase.

This paper describes the methods of preparation of these catalases in the crystalline state.

EXPERIMENTAL

Preparation of crystalline catalase from horse kidney

Six horse kidneys were trimmed of fat and connective tissue and ground. Catalase was extracted from the 3.7 kg of ground kidney with 7.4 liters of distilled water for 4 hours. It was estimated that this extract contained 2.5 g of catalase (Kat. f. 55000), and only 150 ml of blood which could account for only a negligible fraction of the catalase present.

While stirring rapidly, 3.2 liters of alcohol chloroform mixture (3 : 1) were added to the extract. The mixture was allowed to stand overnight in the cold room.

The solution was centrifuged and then filtered so that it was completely clear with a yellow-green color. There is very little loss of activity during the filtration which may take several hours at 20°. The filtrate was then evaporated *in vacuo* to a volume of 360 ml. It was dark brown in color, slightly turbid, and exhibited a distinct catalase absorption band at 625 mμ in a 1 cm layer.

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Acetate buffer (4 *M*, pH 4.0) was added with stirring to bring the final acetate concentration to 0.1 *M*. After several hours at room temperature a brown denatured protein separated out and was removed by centrifugation. This solution was then fractionated with acetone as follows:

To 360 ml was added 188 ml of acetone and the grey-brown precipitate centrifuged off and discarded. Then a further 188 ml of acetone was added to the supernatant solution and the catalase separated as a dark brown precipitate. The yellow supernatant solution, which had no visible spectrum, was discarded.

The precipitate was dissolved in 110 ml of 0.1 *M* acetate buffer pH 4, centrifuged clear, and fractionated with alcohol. To 110 ml was added 84 ml of alcohol and the grey-white precipitate discarded. Upon further addition of 56 ml of alcohol to the supernatant solution a greenish precipitate of catalase was obtained.[8] This was redissolved in a small amount of 0.1 *M* acetate buffer (about 60 ml) clarified by centrifugation, and dialyzed overnight against distilled water.

A sediment appearing during dialysis was discarded, and then ammonium sulfate was added to the solution to make it 0.4 saturated. The brown precipitate was centrifuged off, and more ammonium sulfate slowly added to the supernatant solution to bring the degree of saturation to approximately 0.43. After a few hours the solution became thixotropic and the catalase began to crystallize.

The catalase crystals had the form of fine needles and dissolved readily in distilled water. The yield was 250 mg of crystalline catalase.

Preparation of human liver catalase

Preliminary to a study of catalase distribution in pathological tissues, a method was developed for accurately assaying human livers* for catalase. This method, which will be described in detail in a later publication involved a controlled extraction of the catalase from livers and titration with permanganate. The mean of analyses on 18 normal human livers was 0.61 g of catalase/kg liver. This compares with 0.5 g of catalase/1000 ml of human blood.

The catalase was isolated from human liver by the following procedure.

Two human livers were cleaned of connective tissues and of the larger blood vessels and ground. 3 kg of ground liver was extracted for 4 or 5 hours with 6 liters of distilled water. The amount of catalase in the extract was estimated as 1.8 g (Kat. f. 40000); the blood content 150 ml.

As the kidney preparation described in the foregoing section, the extract is treated with alcohol-chloroform (2.6 liters) and let stand overnight in the cold. Filtration yielded a yellow-green, clear solution with a reddish tint from the cytochrome c present in liver. Upon evaporating in vacuo to 400 ml the absorption bands of catalase (625 mμ) and cytochrome c (550 mμ) were visible.

The pH was adjusted to 4.0 with acetate buffer and the solution fractionated with acetone and alcohol as described above. In this case, 400 ml of solution were used and

* Supplied by Rättsmedicinska Institutet, Stockholm.

the catalase fraction collected between 200 and 360 ml of acetone; the catalase dialyzed for a few hours; and then fractionated with alcohol.

Cytochrome c is usually insoluble after the alcohol fractionation. Any remaining trace, however, was removed by precipitating the catalase at 0.7 saturation of ammonium sulfate.

The catalase solution was dialyzed and crystallized with ammonium sulfate as described for the kidney preparation. The crystals were readily soluble in distilled water. Yield was 275 mg.

PROPERTIES

Measurement of Activity. The catalase activity was estimated as described by v. Euler and Josephson.[7] 0.4 to 0.5 g of catalase were used for each determination. Values of Kat. f. determined in this way are 55000 for kidney catalase and 40000 for human liver catalase. The figures are necessarily approximate, for the extrapolation to zero time is difficult. Preliminary work has indicated a rapid decrease in activity during the first few seconds so that the true Kat. f. may be considerably higher than indicated. This phenomenon may be due to adsorption on the walls of the reaction vessel and is being investigated further.

Hemin and Iron Analyses. Hemin was determined as pyridine hemochromogen. Iron was measured photometrically as the sulfosalicylic acid complex following combustion with H_2SO_4 and »Perhydrol».

	Hemin %	Iron %
Horse kidney catalase	1.030	0.097
Human liver catalase	0.790	0.090

On splitting with conc. HCl and acetone [2] the kidney catalase yields a faint green color in the water phase. The human liver catalase does not give any blue color as the horse liver catalase does, but on standing for about 1 hour in the HCl and acetone solution a faint blue color developes. H_2SO_4 and acetone do not give any coloring of the water phase with human liver or horse kidney catalase, while the horse liver catalase in all cases gives a strong blue coloring of the water phase even with a very low HCl acid concentration (0.1 ml $1N$ HCl + 10 ml acetone).

Absorption Spectrum. The visible absorption spectra as measured with a Beckman spectrophotometer are shown in fig. 1. The Soret band for both has a maximum absorption at 405 mμ where β for kidney catalase is 84.5×10^7 cm^2 μM^{-1} and 67×10^7 for human liver catalase.

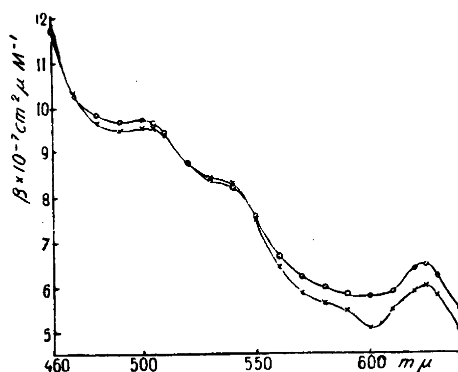

Fig. 1. Absorption spectra. —O— *Human liver catalase 0.0055 µM/ml and* —×— *horse kidney catalase 0.0042 µM/ml. 0.01* M *phosphate buffer pH 6.8, d = 1 cm.*

SUMMARY

Methods have been described for isolating crystalline catalase from horse kidney and human liver, and data presented on the distribution of human catalases in blood and liver. The visible absorption spectrum of the kidney and human liver catalases has been determined. Analyses for hemin and iron have been made.

REFERENCES

1. Sumner, J. B., and Dounce, H. L. *J. Biol. Chem.* **121** (1937) 417.
2. Agner, K., *Arkiv Kemi, Mineral. Geol.* **16** A (1942) no. 6.
3. Laskowski, M., and Sumner, J. B. *Science* **94** (1941) 615.
4. Bonnichsen, R. K., *Arch. Bioch.* **12** (1947) 83.
5. v. Euler, H., Chemie der Enzyme, II : 3. Leipzig (1934).
6. Margulis, S. and Levine, V. E. *J. Biol. Chem.* **41** (1920) 42.
7. v. Euler, H., and Josephson, K. *Ann.* **455** (1927) 1.
8. Agner, K., and Theorell, H. *Arch. Bioch.* **10** (1946) 320.

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