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Received February 19, 1963. P.S.E.B.M., 1964, v115.

Studies on Activation of Intracellular Catalase of Mouse Liver. (28820)

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Recent investigations have indicated that catalase is probably localized within a specific type of particle associated with the mitochondrial fraction. For example, uricase and catalase were found to migrate together during sucrose density gradient centrifugation for 2 hours(1). DeDuve and co-workers(2) also reported that the uricase-containing particle could be separated from lysosomes by means of glycogen equilibration. Catalase activity was found to be associated with the former particle. Furthermore, different particle sites for acid phosphatase and uricase were found after a suspension of the mitochondrial fraction was subjected to sucrose density gradient equilibration(3). Thus catalase was thought to be localized within the so-called uricase particle which differs from both the large mitochondria, site of a variety of metabolic functions including oxidative phosphorylation, and the lysosomes which contain acid phosphatase and other hydrolytic enzymes(4).

The possibility that the particle containing catalase may be surrounded by a membrane through which leakage of this soluble enzyme may occur is indicated by its ease of redistribution following homogenization and isolation of the various subcellular fractions(5,6). The rate-limiting role of this membrane was demonstrated not only by variations in catalase activity of the mitochondrial fraction produced by differences in homogenization, isolation and resuspension procedures but by treatment with detergents (7,8).

Investigations of the behavior of catalase of the mitochondrial fraction* under various conditions of sucrose isolation and suspension were studied and the effect of deoxycholate, phosphate buffer at pH 2 and 1.25 M sucrose on the catalase activity of several cytoplasmic fractions were carried out.

Methods. Preparation of cytoplasmic fractions. Liver from male mice of the IBAH strain was homogenized in 10 volumes of 0.25 M sucrose. The homogenization consisted of 3 strokes at 4500 rpm in a Potter-Elvehjem type homogenizer. The homogenization was carried out over a time interval of 20 to 25 seconds. The homogenates were utilized for isolation of mitochondrial and cell sap fractions according to a procedure previously reported(9).

The mitochondrial and cell sap fractions were also isolated from 0.88 M sucrose. In this case, the liver was again homogenized in 10 volumes of the medium followed by centrifugation at 14,000 $\times$ g for 15 minutes. The supernatant fraction was centrifuged at 21,000 $\times$ g for 30 minutes in rotor #40 (Spinco model L) in order to obtain the mitochondrial fraction.

* The term mitochondria as used in this discussion refers to the mitochondrial fraction as isolated by differential centrifugation from 0.25 M sucrose homogenates. This fraction includes the light and heavy mitochondria as well as lysosomes and uricase particles.

TABLE I. Effect of Sucrose Concentration of Homogenization Medium on Distribution of Mitochondrial and Cell Sap Catalase.

Subcellular fraction*	Homogenization medium	Total catalase activity of fraction $\times 10^3$	% of combined activity†
Mitochondria	.25 M sucrose	2844	87.6
"	.88 M "	2594	74.5
Cell sap	.25 M "	401	12.4
" "	.88 M "	889	25.5

* Mitochondrial fractions were resuspended in .25 M sucrose containing .50% deoxycholate for 15 min before assay in order to produce maximum activity.

† Per cent of activity was based on combined activities of mitochondria and cell sap components for a given homogenate as 100%.

TABLE II. Effect of Deoxycholate, Acidity, and Hypertonic Sucrose on Catalase Activity of Several Cytoplasmic Fractions.

	Catalase specific activity $\times 10^3$			
Subcellular fraction	.25 M sucrose control	.25 M sucrose, .5% deoxycholate	.25 M sucrose, 1 mM $NaPO_4$, pH 2	1.25 M sucrose
Mitochondria	13.5	45.4 (336)*	41.2 (312)	32.0 (237)
Cell sap	9.6	9.4 (98)	9.1 (95)	9.7 (101)

* Quantities in parentheses represent % of control activity.

In a number of experiments, the mitochondrial and cell sap fractions were pretreated with 0.5% deoxycholate, 1.25 M sucrose and 1 mM phosphate buffer at pH 2. The latter solution was prepared by adjusting monobasic sodium phosphate to pH 2 with hydrochloric acid.

Assay procedure. The catalase assay utilized was based on the titration of excess H_2O_2 substrate by $KMnO_4$(10). Aliquots were withdrawn every 15 seconds over an incubation period of one minute carried out at room temperature which varied between 23-25°. The flask contents were titrated to a pink color end-point with a 2.0 ml microbiuret containing 5.0 mM $KMnO_4$ solution. A visible color change could be detected after addition of 0.005 ml of $KMnO_4$ solution. When the catalase rate of reaction represented a straight line, catalase activity could be calculated from the equation for a first order reaction and was expressed as units per second.

Aliquots were taken from each of the subcellular suspensions for protein assay according to the Lowry method(11). Crystalline bovine plasma albumin from Armour Laboratories was used as the standard. The specific activity of the catalase was expressed as units per second per mg protein. In this case, a unit of catalase activity is defined as the amount of hydrogen peroxide decomposed, expressed in terms of ml of permanganate used in titration of the remaining substrate at various incubation times.

Results. When liver is homogenized in 0.25 M sucrose, the mitochondrial and cell sap fractions account for 83.4 and 11.1% of the catalase activity of the homogenate. The remaining fractions comprise only 5.5% of the enzyme activity.†. After homogenization in 0.88 M sucrose, the combined catalase activity of the mitochondrial and cell sap components increased 7% compared to 0.25 M sucrose homogenization and the proportion of catalase in the cell sap increased from 12.4 to 25.5%. Table I depicts the redistribution of catalase between the mitochondrial fraction and cell sap and the overall increase in catalase activity as a result of homogenization in media of different sucrose concentration.

Resuspension of the mitochondrial fraction in hypertonic sucrose solutions or in isolation media containing 0.5% deoxycholate or 1 mM phosphate buffer at pH 2 causes a large increase in catalase activity as shown in Table II. However, similar treatment for cell

† Unpublished data.

TABLE III. Effect of Mitochondrial Catalase Leakage on Activation.

Subcellular fraction*	Resuspension medium	Total catalase activity × 10^3 (units/sec) Mitochondrial suspension	Soluble catalase (I)	Soluble catalase (II)	Residual mitochondria (III)	Total activity (I-II-III)	Mitochondrial suspension (I-II-III)
Mitochondria	.25 M sucrose	184.2	11.7	26.5	174.0	212.2	1.15
"	1.25 M "	400.8	352.0	42.7	81.2	475.9	1.19
"	.25 M sucrose, 1 mM $NaPO_4$, pH 2	523.6	534.0	0	8.3	542.3	1.04

* Mitochondrial fractions were isolated from the same (.25 M sucrose) liver homogenate which was divided into 3 approximately equal fractions, resuspended in different media, and assayed. Then catalase activity of supernatant fractions (I and II) and residual mitochondria (III) were determined.

sap catalase (or solutions of crystalline catalase) produce no effect on the activity. These data indicate that the effect of the above treatment is on the particulate site of the enzyme rather than on catalase *per se*.

When the mitochondrial fraction is isolated from 0.25 M sucrose, resuspended twice in the same medium and both the washings and residual mitochondrial components are assayed for catalase activity, it was found that only a small percentage of this activity was found in the soluble state. Most of the catalase remained associated with the particulate material. When the mitochondrial fraction is resuspended in 1.25 M sucrose or 0.25 M sucrose containing 1 mM sodium phosphate at pH 2, however, the catalase activity increases with a concomitant increase in the amount of soluble catalase as shown in Table III.

The amount of catalase activity associated with the soluble (cell sap) or particulate (mitochondrial) fractions was previously shown to depend upon the sucrose concentration of the homogenizing medium (Table I). The resuspension of the mitochondrial fraction in media of various concentrations of sucrose also affects the particulate catalase activity. Fig. 1 shows the rise in catalase activity of the mitochondria with increased sucrose concentration of the resuspension medium. Simultaneously, the soluble catalase activity (after removal of the mitochondrial fraction by centrifugation) showed an initial drop, at approximately the sucrose concentration used for homogenization and isolation of these particles, followed by a rapid increase paralleling the catalase activity of the original mitochondrial suspension at higher sucrose concentrations. The residual mitochondrial fractions, when resuspended in media of various sucrose concentrations, showed the greatest catalase activity at those sucrose concentrations at which catalase leakage, as measured by soluble catalase, was minimal.

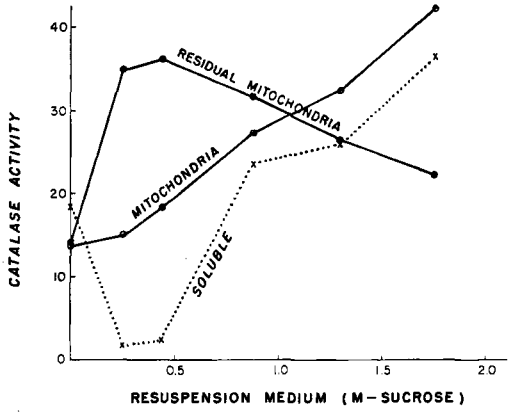

FIG. 1. The mitochondrial fraction was isolated from 0.25 M sucrose, divided into 6 approximately equal fractions and resuspended in media of varying sucrose concentration. The catalase specific activities (units/sec mg protein) of the first and second mitochondrial resuspensions are represented by (○) and (●), respectively, connected by solid lines. Total activity of the catalase which was found in the surrounding medium was determined by assay of the supernatant fraction after centrifugation of the first mitochondrial suspension and is represented by (×) connected by dashed lines.

Discussion. Treatment of the mitochondrial fraction with deoxycholate, phosphate buffer at pH 2 and 1.25 M sucrose results in large increases in catalase activity which cannot be produced with catalase from the cell sap moiety. Changes in the catalase activity

of this particulate fraction are also produced by isolation and resuspension in media of different sucrose concentrations. These phenomena which occur in the mitochondrial fraction but not in the cell sap fraction can best be explained by postulating an effect on the permeability of the membrane of the catalase-containing particle. This possibility has already been offered to explain the effects of the simultaneous solubilization and activation of catalase of the mitochondrial fraction by triton x-100(8). Other investigators(4) have also postulated that the activation of acid phosphatase and other soluble enzymes associated with the lysosome fraction was probably due to damage or disruption of the membrane of this granule.

The data herein presented clearly show the relationship between degree of mitochondrial catalase leakage and activation. When mitochondria isolated from 0.25 M sucrose is resuspended in 1.25 M sucrose, a 218% activation occurs concomitant with a large increase of catalase activity in the surrounding medium (Table III). After a second resuspension and a further increase of soluble catalase activity, 20% of the catalase remains in the mitochondrial fraction. Treatment of the 0.25 M sucrose mitochondria with phosphate buffer at pH 2 which produces maximum catalase activation results in the appearance of virtually all of the catalase activity in the surrounding medium. Therefore, it is proposed that the membrane of the catalase-containing particle of the mitochondrial fraction is the rate-limiting factor in the catalase reaction.

The activation of catalase may not be due exclusively to leakage of catalase from this type of particle. The increase in membranal permeability to H_2O_2 in cases where some catalase still remains within the particle of the mitochondrial fraction may also play a role in the increased activity. The curves in Fig. 1 confirm the relationship between catalase solubility and activation by depicting the parallel between them. They also demonstrate, however, that a second resuspension of the mitochondrial fraction produces higher catalase activities than that of the original mitochondrial suspension at sucrose concentrations below 0.5 M where no great catalase leakage occurred as a result of the first resuspension. This may be explained by an increase of permeability of the catalase-containing particle membrane to H_2O_2 after a second resuspension. Fig. 1 shows, for example, that resuspension of the mitochondrial fraction in water results in a relatively higher catalase activity in the surrounding medium and a lower total activity of the mitochondrial water suspension as compared to suspension in 0.25 M or 0.44 M sucrose. This occurrence may be explained by the lower permeability to H_2O_2 of the residual mitochondrial fraction when resuspended in water. Therefore, maximum activation occurs when all of the catalase has escaped from its particulate site and is in the soluble form as in the case of pH 2 phosphate or deoxycholate treatment. Partial activation, on the other hand, appears to be due to partial leakage of catalase from the particle and to the increased permeability of the membrane to H_2O_2 for the catalase remaining at the particulate site.

Summary. The relative amounts of catalase associated with the mitochondrial or cell sap fractions were influenced by the sucrose concentration of the homogenizing medium. The sucrose concentration of the resuspension medium of the mitochondrial fraction was found to affect catalase activity. The different response of mitochondrial and cell sap catalase to deoxycholate, phosphate buffer at pH 2 and 1.25 M sucrose treatment was ascertained. The increase of mitochondrial catalase activity when pretreated with pH 2 phosphate, deoxycholate and 1.25 M sucrose could be explained by the influence of these agents on the permeability of the membrane enveloping this enzyme. Maximum activation of the particulate catalase is concomitant with complete leakage of the enzyme from the particle site. Partial catalase activation is probably due to a combination of leakage and increased permeability of the membrane to H_2O_2 substrate.

This author is indebted to Dr. Anna Goldfeder for her guidance and interest during this investigation. This work was supported by Nat. Cancer Inst., Dept. HEW, U. S. Public Health Service.

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Received May 10, 1963. P.S.E.B.M., 1964, v115.

Effect of Variations in Sodium Intake on Calcium Excretion in Normal Humans.* (28821)

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The amount of calcium excreted per unit time represents the balance between the quantity filtered at the glomerulus and the magnitude of reabsorption by the renal tubules. The filtered load is the product of the concentration of diffusible (complexed and free Ca^{++} ion) calcium in the plasma and the glomerular filtration rate, while tubular reabsorption appears to be an active transport process which takes place in both proximal and distal parts of the nephron(1). There is no evidence that net tubular secretion of calcium occurs(1). The relative importance of alterations in filtered load or tubular reabsorption to the homeostatic regulation of calcium excretion has not been defined. However, it is clear that normally greater than 95% of the filtered calcium is reabsorbed. The latter process seems to be influenced by the level of the filtered load (2,3), the concentration of parathyroid hormone in body fluids(3), the overall calcium balance or body "stores," the quantity of organic and inorganic anion which may complex free Ca^{++} ion in luminal fluid, and the nature of the other univalent and divalent cations simultaneously excreted(2,4,5,6).

Walser(6) has conclusively demonstrated in the dog a direct relationship between the renal clearances of calcium and sodium. The clearances of these 2 ions were remarkably similar, and any increase in sodium excretion immediately caused an increase in calcium excretion. It is quite obvious that this relationship could greatly effect the interpretation of the homeostatic regulation of calcium excretion in any clinical or experimental study.

The present study was undertaken to determine whether these observations in the dog could be confirmed in the human.

Methods and procedure. The experiments were carried out on 6 normal young adult volunteers ranging in age from 25 to 35. All were males except subject M.K. Throughout the study each subject was maintained on a near-constant calcium intake by eating the same quantity of dairy foods and leafy vegetables daily. The only significant variable in the diet was the intake of sodium chloride. Twenty-four hour urinary collections were made by each subject while carrying out his normal daily activities. The sodium content of the diet was varied from 20 to 425 mEq/24 hours by addition of weighed quantities of NaCl to diets calculated to contain from 200 mg to 2 g of sodium. Each subject re-

* Supported by USPHS grant.