# ON THE INTRACELLULAR LOCALIZATION AND BIOSYNTHESIS OF CATALASE IN LIVER TISSUE

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GREAT deal of doubt exists in the literature regarding the intracellular distribution of catalase in the liver tissue. Euler and Heller[1] found only 16-23% of the total enzyme in the mitochondrial fraction. Ludewig and Chanutin,[2] however, observed about 45% in the mitochondria and Greenfield and Price[3] reported that 63% of the catalase could be recovered in the mitochondrial fraction provided that Polyvinyl Pyrrolidone was added to prevent the elution of the enzyme into the cell sap. Thomson and Klipfel[4] observed that catalase and uricase sediment closely together during gradient density centrifuging, in a particulate fraction lighter than the particles containing the succinic dehydrogenase and cytochrome oxidase. de Duve *et al.*[5] have observed a close relationship in the sedimentation properties of the particles containing uricase, catalase and D-amino-acid-oxidase and suggested that these enzymes are present in the subcellular particles distinct from both mitochondria and lysozymes.

The claims of the earlier investigators on the intracellular distribution of catalase using the Perfused rat liver tissue have been re-examined. 0·25, 0·33 and 0·88 M sucrose solutions were used as the homogenizing media and the technique of Schneider *et al.*[6] was adopted. In all cases it was found that about 60% of the catalase activity was present in the 100,000 × g. supernatant fraction. We could demonstrate the elution of catalase from the mitochondrial fraction when only 0·25 M sucrose solution was used for washing the mitochondria. Such an elution of catalase activity from mitochondria was considerably prevented when 0·88 M sucrose solution was used. Hence 0·88 M sucrose solution was selected for the study on the distribution of catalase in the liver tissues of mouse, rat, guinea-pig, rabbit, sheep and monkey. The catalase activities in the fractions were estimated by the titrimetric method of Euler *et al.*[7] as described by Radhakrishnan *et al.*[8] The protein content of the fractions was determined by the method of Lowry *et al.*[9] The results presented in Table I reveal that in all the species tested, the catalase activity is present mainly in 100,000 × g. supernatant fraction.

In view of the reports[10,11] on the alterations of catalase activity in the livers of hyper and hypothyroid rats, experiments were undertaken to examine, in which subcellular fraction of the rat liver tissue, the catalase activity is affected under such a dietary condition. Young male albino rats weighing about 80–90 gm. were divided into three groups of six and the first group served as the control group (A) being maintained on a synthetic diet as described by Rajalakshmi *et al.*[12] The fat-soluble vitamins were supplied by mixing 2 to 3 drops of Adexolin (Glaxo Laboratories, Bombay, containing 12,000 I.U. of Vitamin A and 2,000 I.U. of Vitamin $D_2$ B.P. per gramme) in the diet twice in a week. In addition to the stock diet as supplied to the rats belonging to the first group (A) the second group of rats (B) were given each intraperitoneal injections of 0·5 mg. of l-thyroxine on alternate days. The diet given daily to the third group of rats (C) was essentially the same as that of control rats excepting that it contained 50 mg. of 2-thiouracil in addition. All the rats were given food *ad lib*. The weights of the rats were recorded at the end of every week and the average growth rates of rats belonging to the three groups are given in Fig. 1. At the end of the

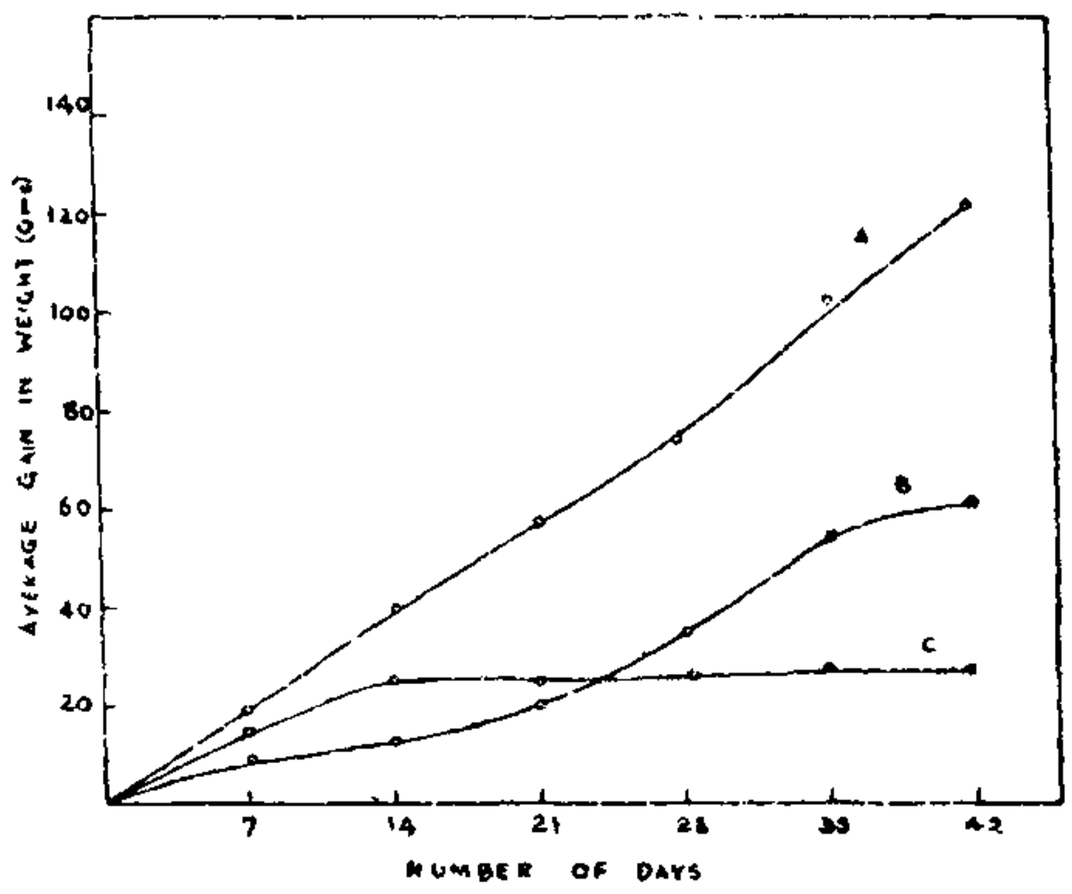


FIG. 1. Growth rate of control, hyper and hypothyroid rats. A, B and C represent the growth curves of control, hyper and hypothyroid rats respectively.

sixth week, the animals were killed and the catalase activity in the blood and in the subcellular fractions of the liver tissues was estimated. The results summarized in Table II show that the catalase activity in the super-

TABLE I

*Intracellular distribution of catalase in liver tissues of various animals*

| Fraction | Mouse | | | Rat | | | Guinea-pig | | |
|---|---|---|---|---|---|---|---|---|---|
| | Protein mg. | % Protein | % Catalase activity | Protein mg. | % Protein | % Catalase activity | Protein mg. | % Protein | % Catalase activity |
| Whole Hon ogenate | 126·0 | 100·0 | 100·0 | 137·0 | 100·0 | 100·0 | 144·0 | 100·0 | 100·0 |
| Nuclei .. | 15·3 | 12·1 | 5·0 | 18·0 | 13·0 | 10·0 | 15·0 | 10·4 | 1·0 |
| Mitochondria .. | 21·2 | 16·8 | 35·0 | 34·0 | 25·0 | 23·0 | 20·2 | 14·0 | 9·0 |
| Microsomes .. | 18·5 | 14·7 | 10·0 | 21·0 | 16·0 | 8·0 | 16·9 | 11·7 | 3·0 |
| Supernatant .. | 50·7 | 40·2 | 47·0 | 60·0 | 44·0 | 58·0 | 66·0 | 45·8 | 83·0 |
| TOTAL .. | 105·7 | 83·8 | 97·0 | 133·0 | 98·0 | 99·0 | 118·1 | 81·9 | 96·0 |

| Fraction | Rabbit | | | Sheep | | | Monkey | | |
|---|---|---|---|---|---|---|---|---|---|
| | Protein mg. | % Protein | % Catalase activity | Protein mg. | % Protein | % Catalase activity | Protein mg. | % Protein | % Catalase activity |
| Whole Homogenate .. | 125·0 | 100·9 | 100·0 | 92·5 | 100·0 | 100·0 | 168·0 | 100·0 | 100·0 |
| Nuclei .. | 18·0 | 14·0 | 8·0 | 11·9 | 12·8 | 6·0 | 23·4 | 13·9 | 8·0 |
| Mitochondria .. | 29·0 | 23·0 | 15·0 | 13·2 | 14·3 | 11·0 | 24·2 | 14·4 | 17·0 |
| Microsomes .. | 20·0 | 16·0 | 7·0 | 13·7 | 14·8 | 3·0 | 30·9 | 18·3 | 9·0 |
| Supernatant .. | 53·0 | 44·0 | 68·0 | 55·0 | 59·5 | 77·0 | 82·8 | 49·2 | 66·0 |
| TOTAL .. | 120·0 | 97·0 | 98·0 | 93·8 | 101·4 | 97·0 | 161·3 | 95·8 | 100·0 |

The catalase activity was calculated in terms of ml. of 0·1 N potasium permanganate equivalent to hydrogen peroxide consumed per minute per mg. protein. The relative activities are expressed as percentage activities of the whole homogenates.

TABLE II

*Influence of hyper and hypothyroidism on the catalase activities in the subcellular fractions of rat liver tissue and in blood*

| | Fraction | % Change in catalase activity* | |
|---|---|---|---|
| | | Hyperthyroid rats | Hypothyroid rats |
| Liver† .. | Whole homogenate | −25·0 | −42·0 |
| | Supernatant | −29·0 | −37·0 |
| Blood .. | Erythrocytes | +20·0 | +16·0 |

* The catalase activities calculated as mentioned under Table I are expressed as % change over the control rats. The figures represent the average of six values.

† The nuclear, mitochondrial and the microsomal fractions of the liver tissues showed no change in the catalase activity.

natant fraction alone was affected under these dietary conditions. However the blood catalase activity was found to have increased slightly in both hypo and hyperthyroid animals.

In view of the above observations on the intracellular localization of liver catalase, the incorporation of $Fe^{59}$ into catalase has been studied. The preliminary results obtained in the *in vivo* and *in vitro* experiments with rats revealed that only the catalase isolated from the supernatant fraction of the liver tissue contained the maximum $Fe^{59}$ activity. When the subcellular distribution of $Fe^{59}$ in 'Heme' was measured it was found that only the mitochondrial fraction exhibited the maximum radioactivity. Further, in view of the presence of Heme' synthetase[13,14] in mitochondria, it is possible that mitochondria has a role in the biosynthesis of the prosthetic group of catalase. The results obtained in support of this concept will form the subject-matter of a detailed communication to be published elsewhere.

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## INTERNATIONAL SYMPOSIUM ON CONTINUUM MECHANICS

THE importance of Continuum Mechanics to bridge the gulf between microscopic and macroscopic description of natural phenomena is now widely recognised. It has received attention from a large number of prominent workers all over the world. The International Union of Theoretical and Applied Mechanics (IUTAM) could not have done better than to hold a Symposium on this subject at Tbilisi, Georgia, U.S.S.R., from September 17th to 23rd, 1963.

The analytical theory of functions plays a vital role in the mathematical description of continuum problems. It was, therefore, in the fitness of things that the symposium was held under the Chairmanship of Professor N. I. Muskhelishvili, President of the Georgian Academy of Sciences, who along with his co-workers has contributed a lot to the applications of the Theory of Functions of Complex Variables to problems in mechanics.

Participation in the conference was by invitation and 61 foreign workers and 55 Russian workers attended it. 20 countries were represented and 70 research papers were presented, which included six general lectures.

The general lectures reviewed the work of various schools. N. I. Muskhelishvili dealt with Elasticity and L. I. Sedov with Hydrodynamics. I. N. Sneddon, F. G. Tricomi, S. Bergman, L. A. Galin gave applications of Integral Equations. Non-linear problems were treated by B. R. Seth, Z. N. Dobrovol'skaya, A. A. Dorodnitsyn and J. P. Germain. A number of papers dealt with contact problems.

The spectral analysis of continuum phenomena can be split into two parts—linear and non-linear. The middle part of the spectrum is generally linear and has been extensively dealt with. But the transition from the linear to non-linear at the ends of the spectrum has not received sufficient attention. This involves the determination of asymptotic solutions at the turning points of differential systems. The theory of analytic functions of one or more complex variables whose use was extensively illustrated at the conference can be further extended to asymptotic solutions. Another modern trend to which attention should be drawn is the reduction of solutions of problems to integral equations, which can be readily solved on high speed digital computers. In fact, one such problem, arising out of the formations of small waves on a non-uniform bed, was suggested by the digital computer. It is unfortunate that in India, we are still not very conscious of the importance of high speed computers as a powerful tool to solve scientific problems. It is hoped that the start made in such computation at Bangalore, Kharagpur, Kanpur and Bombay will spread to many other centres in the country. The problem of asymmetrical stress distribution due to internal stress couples was discussed in two papers. Such situations arise on the microscopic level, and hence the treatment of such problems brings the two fields closer together. Thin and shallow shell problems were treated by a number of Russian workers. Magneto-hydrodynamical problems attracted few participants. In such cases a disturbing effect is observed about the vanishing and reversal of lift, if the external magnetic field reaches certain critical strength in relation to the flow velocity. Attention was also drawn to problems in which the main difficulty lies in the fact that the regions in which the solution is sought is unkown. It was also shown that a number of problems like flow past cascades of profiles and the bending of plates weakened by an infinite set of periodical holes can be reduced to problems for automorphic functions.

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