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ENVIRONMENTAL STRESS

AND

BIOCHEMICAL ADAPTATION

DEPARTMENT OF BIOCHEMISTRY
INDIAN INSTITUTE OF SCIENCE
BANGALORE 560 012

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The regulation of mitochondrial succinate dehydrogenase under conditions of environmental stress and treatment with diverse class of activators, including ubiquinol, with the common feature of 5.4+0.8 A have been studied which led to the appreciation of the importance of this enzyme and redox status of ubiquinone in regulation of energy metabolism.

The combined action of the two stress hormones, cortisol and noradrenaline,

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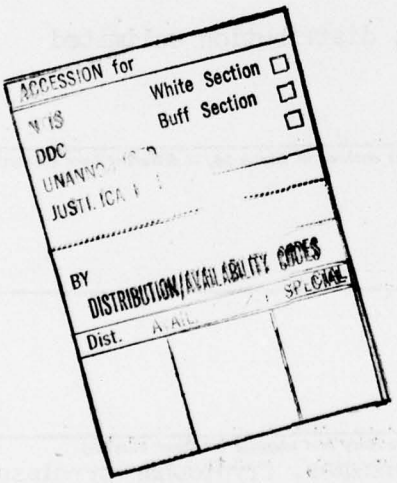
stabilizes the new steady-state levels of the enzymes in the catabolism of aromatic amino acids--tryptophan pyrrolase, tyrosine aminotransferase and phenylalanine hydroxylase.

The observations that enzyme induction and body temperature decreased on treatment with some drugs under cold-exposure point to the need of qualification of environmental conditions for specifying drug action.

Based on these findings models for chemical thermogenesis, acclimation process and functional role of protein structure have been proposed which are expected to stimulate further experiments.



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ENVIRONMENTAL STRESS AND BIOCHEMICAL ADAPTATION.

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ENVIRONMENTAL STRESS AND BIOCHEMICAL ADAPTATION

ABSTRACT

1. Environmental chambers simulating conditions of altered pressure and temperature have been designed and fabricated.

2. The regulation of mitochondrial succinate dehydrogenase under conditions of environmental stress and treatment with diverse class of activators, including ubiquinol, with the common feature of 5.4 ± 0.8 Å have been studied which led to the appreciation of the importance of this enzyme and redox status of ubiquinone in regulation of energy metabolism.

3. The combined action of the two stress hormones, cortisol and noradrenaline, stabilizes the new steady-state levels of the enzymes in the catabolism of aromatic amino acids - tryptophan pyrrolase, tyrosine aminotransferase and phenylalanine hydroxylase.

4. The observations that enzyme induction and body temperature decreased on treatment with some drugs under cold-exposure point to the need of qualification of environmental conditions for specifying drug action.

5. Based on these findings models for chemical thermogenesis, acclimation process and functional role of protein structure have been proposed which are expected to stimulate further experiments.

PREFACE

It is interesting to recall that in 1970 a letter from Dr. L. M. Libber of Office of Naval Research, U.S.A. enquiring about our interest to submit them a research proposal is the beginning of this project. The project got activated in September 1971, thanks to the speedy handling by the UGC.

The purpose set out was to develop facilities to simulate changed environmental conditions of pressure and temperature and to study the alterations of cellular enzymes during environmental stress leading to adaptation at biochemical level. These are largely achieved.

The major thrusts are: The study of regulation of mitochondrial succinate dehydrogenase which lead to the models for thermogenesis and caloric redistribution; The hormonal controls of inducible enzymes in the catabolism of aromatic amino acids which helped in hierarchical modelling of acclimatory processes; The theme of modification of drug action and the caution needed to check drops in body temperature; The theoretical study on the secondary structure of proteins - the π-H pathways - that may offer an explanation for thermogenesis at molecular level and lead to study on the understanding of protein functions. While the work lead to some information on how the changes take place under environmental stress, the basic question of what determines the steady state in a cell to suit an environment remains open.

The work was carried out by my 8 colleagues - 3 at post-doctoral level, 5 graduate students 4 of whom already used the work for their Ph.D. theses. The credit of the experimental work is theirs. The devotion of the supporting staff also deserves mention. Cooperation of the staff from other departments provided some inter-disciplinary character to the project. In general, interaction with other members of my group and other projects in the laboratory has contributed in a two-way improvement of the work.

The staff and I had participated in 58 Symposia/Seminars - 7 Symposia of international character, 15 national symposia and 36 seminars - and presented the work which sharpened the ideas during the progress of the project.

The work was published in 26 papers comprising of 5 short communications, 11 full papers and 10 symposia papers. Five more full papers are being processed.

It has been a pleasure to have the association of Dr. Libber who gave constant encouragement and thoughtful review of the progress report each year. My thanks are due to Dr. S. Dhawan, Director, who gave support in sponsoring this project from the Institute.

T. Ramasarma

Bangalore
May 1, 1977

(T. RAMASARMA)
Principal Investigator

ENVIRONMENTAL STRESS AND BIOCHEMICAL ADAPTATION

Sept 1971 - April 1977

Research Project

Environmental stress invokes compensatory metabolic changes by modification of quality and quantity of enzymes that are normally rate-limiting or under fine control or inducible by hormones. Cellular enzyme changes in animals exposed to low temperature and pressure conditions form the basis of this investigation. Towards this objective, a four-component environmental simulation chambers for alterations in pressure and temperature have been designed and fabricated (see inside back cover). The experimental approaches are three-fold: oxidative metabolism, catabolism of aromatic amino acids and alteration in drug action. A molecular basis of chemical or non-shivering thermogenesis using shunt pathways of electron transport to divert energy towards heat production had been developed. Exposure of intact animals to low pressure and temperature elicited enzyme changes in the catabolism of tryptophan, tyrosine and phenylalanine which are shown to be under the control of cortisol and noradrenaline. Effects of drugs are altered or abolished indicating modified metabolic responses under conditions of environmental stress. The processes of adaptation at the cellular level to environmental stress seem to occur by sequential changes of hormones, enzymes and metabolites leading to a new steady-state.

SUCCINATE DEHYDROGENASE

The mitochondrial inner-membrane enzyme, succinate dehydrogenase, occupies a central position in energy transformation process by being part of the Krebs cycle and electron transport chain. Thus modulation of its activity will determine both the amount of NADH generated by Krebs cycle and rate of overall respiration. On activation of succinate dehydrogenase, one would expect, an increased turnover of Krebs cycle and thereby increased NADH production. The activation of the very same enzyme will regulate the oxidation of NADH by electron transport chain since the two dehydrogenases compete for the limiting cytochrome system (Fig. 1). This could result in increased availability of NADH for synthetic processes or its reoxidation by non-phosphorylating systems in cellular organelles leading to heat production. Thus the study of modulation properties of this enzyme under conditions of environmental stress had been undertaken. The information obtained in this investigation showed for the first time that this enzyme does indeed respond to the stimuli and alters its activity levels

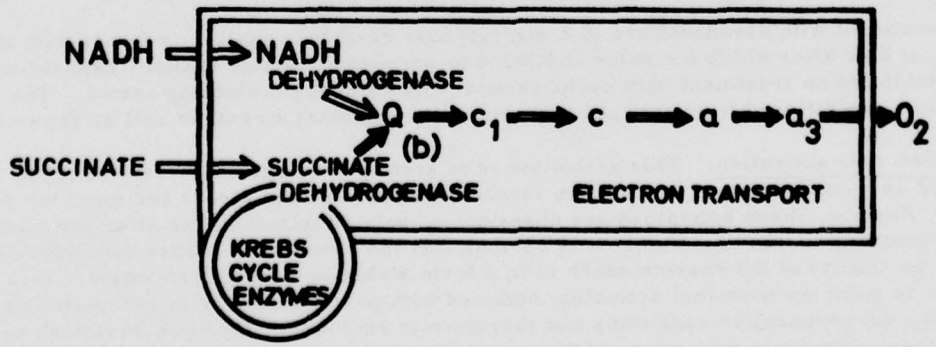


Fig. I Location of succinate dehydrogenase in Krebs cycle and electron transport chain

confirming that the regulation known to occur in isolated state in vitro is possible under conditions in vivo.

Activation of succinate dehydrogenase in vivo

Exposure of rats to hypobaria (0.5 atmosphere) or hypoxia (10% O_2) for short periods resulted in an increase of hepatic mitochondrial succinate dehydrogenase activity. That this increase was due to activation of the preexisting protein was indicated by obtaining the same maximal activity in both control and exposed groups of animals on activation by preincubation of such mitochondrial samples with succinate - a known phenomenon, and also by the failure to prevent the changes on treatment of the animals during exposure to stress with protein-synthesis inhibitor, cycloheximide.

The increase in activity was progressive with time of exposure and reached a maximum by 4 hr and was maintained at this high level under hypoxia for 36 hr but reverted to the basal level under hypobaria by 12 hr (Fig. 2). On withdrawal of the stress at 4 hr, the activity reverted to the basal in 6-12 hr.

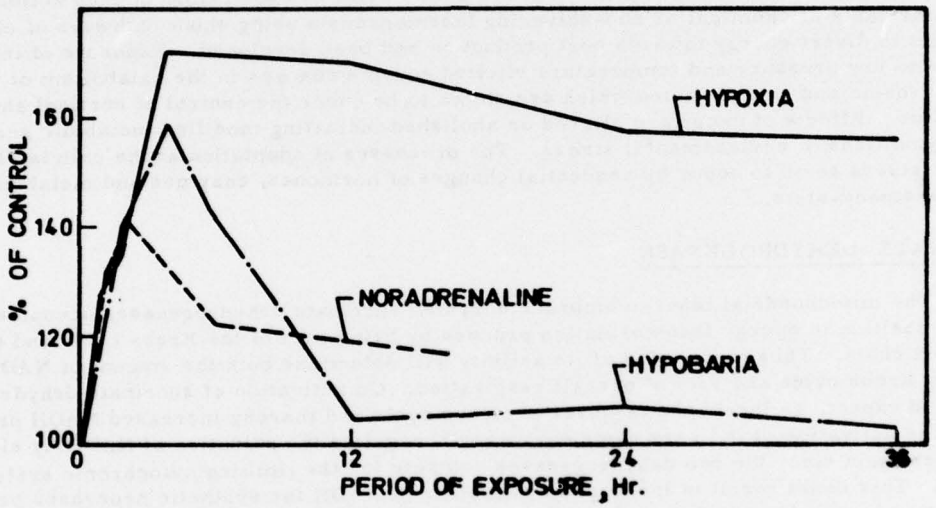


Fig. 2 Activation of hepatic mitochondrial succinate dehydrogenase in vivo

Treatment with noradrenaline (0.2 mg/rat) also elicited a similar response with the maximum at 2 hr after which the value continued to decrease. Noradrenaline response was partially inhibited on treatment with cycloheximide and adrenergic blocking agents. The nature of activation also differed from that obtained with environmental stress as will be shown later.

"KV"-type activation: This activation is of great physiological interest as it can be interrupted as a compensatory mechanism invoked to overcome the stress and meet the energy demands. Further, these activators are observed in isolated mitochondria after two washes with homogenizing sucrose medium. It is obvious that the stimulus of stress had evoked a change in the quality of the enzyme which is in a form stable to washing procedure. It is instructive to point out here that activation obtained with preincubation with succinate is not stable under the preparative conditions and therefore is excluded. These observations promoted further probes into the nature of the activation phenomenon. The first signs of an intricate mechanism is revealed in the experiments to determine the Michaelis constants. It was found that both with hypobaric- and hypoxic-type of activations the double reciprocal plots

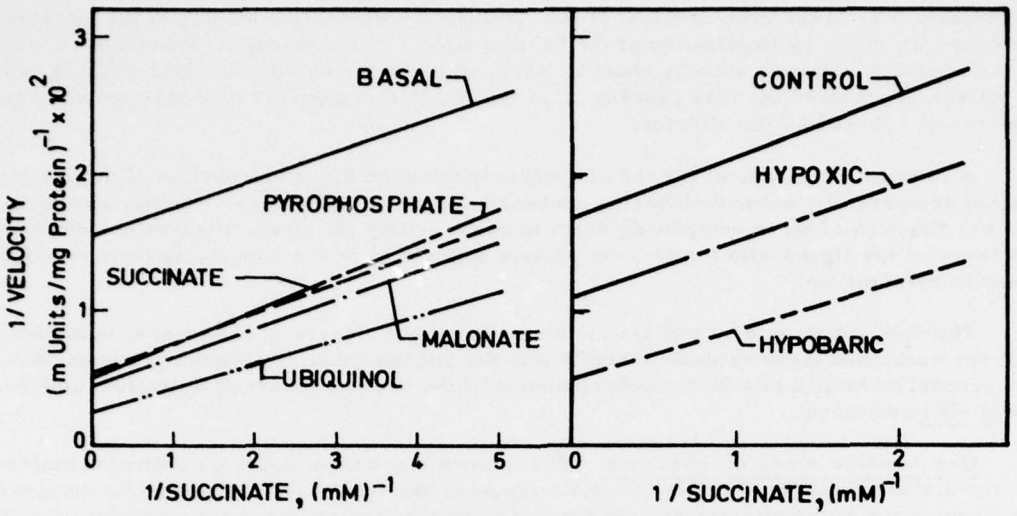


Fig. 3 Activation of succinate dehydrogenase : Lineweaver-Burk plots

(Fig. 3) were parallel to that of the basal showing both K_M and V_{max} were altered in the activated enzyme. There was sigmoidicity with [S] versus V curves. The same was true of the succinate-activated enzyme giving the clue that a general phenomenon of activation distinct from the classical "K" or "V" - types is under observation.

It was conjectured that the activation phenomenon represents a conformational change of pre-existing protein into a form with higher catalytic efficiency. A number of activators were known by the early seventies and the work had taken an unexpected turn when it became known that activators had invariably the property of competitive inhibition at higher concentrations. It was considered useful to distinguish the activators, even if it were to be some minor property. And towards this end some of the features of activation were investigated in detail.

Some features of activation: The assay of the enzyme is normally carried out by the rate of reduction of the dye, phenazine methosulfate (PMS). This activity is considered to reflect the activity of this flavoprotein, as it interacts with the main-chain electron transport. The same flavoprotein can also be measured by the reduction of the dye, neotetrazolium chloride (NT). The NT-reductase is specifically stimulated by enriching mitochondria with endogenuous or exogenous ubiquinone. The PMS-assay is insensitive to added ubiquinone in vitro and antimycin A whereas the NT-assay is stimulated by ubiquinone and inhibited by antimycin A. The two assays permitted distinction between some of the activators.

The substrate, succinate, itself acts as an activator when preincubated with mitochondria in vitro. By simple washing procedure it can be removed and the resedimented mitochondria reverted to basal activity. Some conditions of activation were stable to such washing procedure. A second washing procedure was adopted in which the mitochondria were first preincubated with excess of succinate for full activation and displacement of any bound activator was brought about by the usual washing procedure. This procedure reverted the activity to basal level in most cases and only those activators that can not easily be displaced by succinate retained the higher activity. In addition, the values of Michaelis constant for succinate (K_M), the concentration of the activators for self-saturation (K_a) and the inhibitor constant (K_i) were experimentally determined.

The Classes of activators: A widely differing type of compounds including substrate competitors have been found to show activation of succinate dehydrogenase. These fall into the classes of dicarboxylates, pyrophosphates, quinols and nitrophenols. To this group are added dihydroxy-phenyl compounds, represented by noradrenaline. It had become axiomatic that a competitive inhibitor by implication should activate the enzyme on preincubation of

mitochondria with lower concentration of the compound. While firm binding of the effector to the enzyme protein - by implication at the "active site" - is indicated, it is difficult to visualize how the enhanced catalytic activity could be realized if the active site was blocked. The finding that activation required far less concentration than inhibition gave the clue that probably not all the sites are covered by the effector.

A common requirement for the activation is the need for preincubation of mitochondria at higher temperature and with differing concentration of the activators. Neither oxidation nor any other reaction of these compounds seem to occur. Only physical, temperature-dependent association of the ligand with the enzyme protein appears to be the underlying feature of the activation phenomenon.

The detailed structural and kinetic studies of these classes of activators, with one example for each, had given valuable insights into the phenomenon. As expected comparison with these properties helped in a better understanding of the possible cause of activation under conditions in vivo examined.

Comparative study of effectors: The factors that characterize an activator molecule are: the distance between the twin ionizable oxygens, the fractional charges on the oxygens, the concentration of the effector and the time of preincubation required for maximum activation. Modification of K_M and reversibility on washing gave additional information. The activated forms of the enzyme obtained on preincubating mitochondria with the effectors exhibited Michaelian kinetics and gave double reciprocal plots which are nearly parallel to that of the basal form as in the case of experiments with the intact animals described before (Fig. 3). The effectors activated the enzyme at low concentrations and inhibited, in a competitive fashion, at high concentrations. The binding constant for activation was lower than that for inhibition. The exception to this is noradrenaline, the activation of which was of the "V" type with K_m for succinate being unaltered. This activation was slow requiring several minutes at 37°C, was partially reversible after washing indicating strong binding, and required higher than physiological concentrations of the activator. The known presence of monoamine oxidase on the mitochondrial outer membrane may explain the requirement of high concentration of this amine. A degradation product of the amine may be the true activator as a lag was observed in the activation by noradrenaline. Search for other dihydroxyphenyl derivatives as potential activators singled out dihydroxybenzoate for this purpose.

The activators have one significant common feature. They possess two ionizable oxygens separated by a distance of 5.5 ± 0.8 Å (Fig. 4) and having fractional charges in the range of

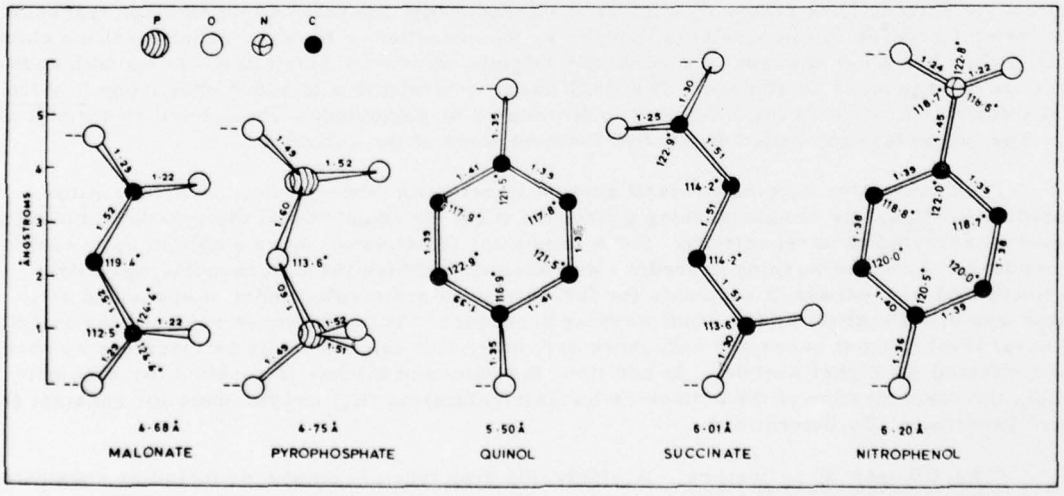


Fig. 4 Three dimensional view of the effectors depicting the twin ionizable oxygens (O_1 - O_2) arranged vertically in a plane along the scale indicated. The O-O distances are shown for each effector. Hydrogen atoms are omitted.

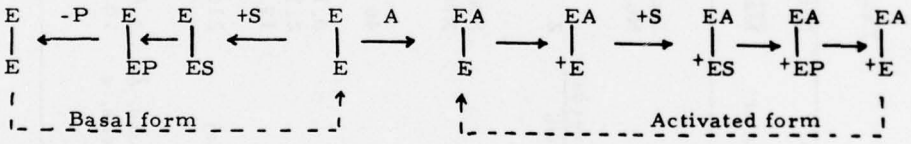
0.26 to -0.74 e. The common twin-oxygen feature of the substrate and the effectors first gave a rational explanation of the diversity of the compounds and suggested the presence of corresponding counter charges in the binding domain, conjectured to be lysines held on α-helix to fit the distance and charges. The competitive nature of effectors with the substrate for inhibition further indicated the close structural resemblance of the activation and catalytic sites'.

The comparative study of the properties of the effectors is given in Table I.

Comparative study of tissues: The foregoing studies were carried out with liver tissue. It was of interest to study the general phenomenon of activation of succinate dehydrogenase in other tissues under conditions in vitro and the response to environmental stress. These studies showed activation of isolated mitochondria on incubation with different effectors and the properties paralleled that of the liver with some notable differences. However, the in vivo responses are specific to the liver. These results are summarized in Table 2.

Comments on the mechanism of activation: The important aspect to consider is that the low-activity protein binds to substrate, or some analogues with twin oxygens, and the conformation is modified with concomitant increase in catalytic activity. Therefore there are two processes - the activation and the catalysis both influenced by the substrate. A ping pong mechanism is to be considered where parallel double reciprocal plots are obtained. But this mechanism requires two substrates and two products with the second substrate being added after the first product is released. This being not the case, the activated enzyme must be considered to represent a different form of enzyme and does not oscillate between two or more stable forms during the reaction as required in the ping-pong mechanism.

The classical Michaelian kinetics and negative co-operativity are characteristic of half-of-the-sites reactivity in which two identical protomers participate in a flip-flop style each, interacting with the substrate. Both "activation" and "catalytic" sites use succinate albeit the catalytic site, more specifically. Compounds that compete with substrate for the catalytic site also interact at the activation site. These observations point out that the two sites, if not identical, must have common structural features. The enzyme, succinate dehydrogenase, is known to be a dimer and it is possible that the first site acts as the "activation site" inducing the second one to assume the catalytic role. Interaction of succinate at the active site increased K_M implying a kind of negative co-operativity. Both sites are interacting with the substrate competitors. At low concentration activation is obtained and at high concentration wherein both sites are blocked, the enzyme activity is inhibited. The reaction is illustrated below:



A Activator
S Substrate
P Product

E Enzyme
+E Activated enzyme
Enzyme is shown in dimeric form

A strong possibility exists that this enzyme has half-of-the-sites reactivity proof for which requires the evidence of existence of the various enzyme forms. Half-of-the-sites reactivity is being increasingly recognized for a number of enzymes having subunit structure and Michaelian kinetics. The additional properties of negative co-operativity, parallel double reciprocal plots and activation by lower concentration of competitive inhibitors, hopefully, may become acceptable criteria for this mechanism. An enzyme with this mechanism possesses a subtle regulatory potential whereby the active form of the enzyme is rapidly obtained on arrival of the substrate and it reverts to a dormant form after exhaustion of the substrate without recourse to energetically expensive protein turnover.

Table I. Comparative study of activators

	Class:	Dicarboxylates	Pyrophosphates	Quinols	Nitro-phenols	Substrate	Dihydroxy-phenylates
	Activator:	Malonate	Pyrophosphate	Ubiquinol	Dinitro-phenol	Succinate	Noradrena-line
I.	Activity						
	PMS reduction	Increased	Increased	Increased	Increased	Increased	Increased
	NT reduction	No change	Increased	No change	Increased	Increased	No change
II.	Conditions of activation and reversal						
1.	Time for maximum activation, min (37°C)	2	Fast	2	Fast	7	8-10
	Reversibility on washing						
	- without succinate	Stable	Partial	Stable	Partial	Reversed	Stable
	- with succinate	Reversed	Reversed	Stable	Reversed	Reversed	Reversed
III.	Kinetic constants						
1.	Apparent K_m (10^{-5}M)	46	48	81	57	42	37
2.	Binding constants						
	Ka	0.14	2.9	6	46	8	620
	Ki	2.7	5.0	-	150	-	170
	Ki/Ka	19.3	1.7	-	3.2	-	0.26
3.	*Vmax (nmol/min/mg protein)*	210	222	413	211	208	167
IV.	Structural features						
1.	Distance between twin oxygens, Å	4.68	4.75	5.50	6.20	6.02	-
2.	Fractional charges on oxygens, e	-0.62	-0.37	-0.73	-0.26, -0.73	-0.62, -0.67	-

Table 2. Comparative study of tissues for activation of succinate dehydrogenase

Effector	Brown Adipose	Heart	Brain	Kidney	Liver
Succinate, malonate, ubiquinol, dinitrophenol, ATP, noradrenaline	Increased	Increased	Increased	Increased	Increased
Mg^{++} requirement for ATP activation	None	None	Required	None	None
Reversal of activation with succinate, malonate and ATP	Reversed	Reversed	Reversed	Reversed	Reversed
Reversal of activation with ubiquinol	Reversed	Reversed	Not reversed	Not reversed	Not reversed
Hypobaria	-	No change	No change	No change	Increased
Hypoxia	-	No change	No change	No change	Increased
Noradrenaline treatment	-	No change	No change	No change	Increased
PMS-reduction					
- ubiquinone	No change	No change	No change	No change	No change
- ubiquinol	Activated	Activated	Activated	Activated	Activated
NT-reduction					
- ubiquinone	Activated	Activated	Activated	Activated	Activated
- ubiquinol	No change	No change	No change	No change	No change

CATABOLISM OF AROMATIC AMINO ACIDS

Increased supply of carbon pool to meet the energy demands under conditions of environmental stress seem to be occurring by the action of hormonal interplay. Catabolism of aromatic amino acids, subject to control by cortisol and noradrenaline, the two "*stress* hormones", seems to provide a reflection of this condition indicating increased gluconeogenesis. The importance of tyrosine and tryptophan is enhanced in view of their precursor role for the neurohormones noradrenaline and serotonin. The concentration of these amino acids in the pool and the hormones will regulate the flow of these into various metabolic routes schematically represented in Fig. 5.

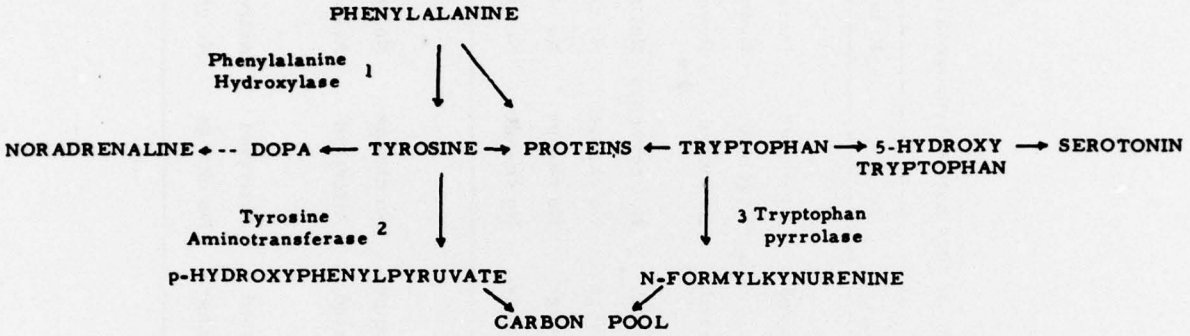


Fig. 5 Metabolic Routes of Tryptophan and Tyrosine

The enzymes tyrosine aminotransferase (I) and tryptophan pyrrolase (2) are the degradative enzymes and are known to be under control of corticosteroids. The catabolism of phenylalanine is effected by converting it to tyrosine by the hepatic enzyme phenylalanine hydroxylase (3), now shown to be under control by cortisol. The two conditions employed were cold exposure and hypobaria. The enzymes increased in a time-dependent fashion and seem to be regulated by the stress hormones acting in some cases via the adrenergic receptors.

Nature of induction in cold exposure: When rats were exposed to cold (0-5°C), the activities of hepatic tyrosine aminotransferase and tryptophan pyrrolase increased rapidly and reached a maximum of 3-fold in 8 hr. On continued exposure upto 48 hr stress, the activity partly decreased but remained at a level higher than control (Fig. 6). Withdrawal from the cold stress reversed the change. A temperature lowering of 7-9°C from ambient was sufficient to show significant increase in the enzyme activity during cold stress indicating a possible involvement of corticosteroids and de novo protein synthesis. Treatment with drugs known to block autonomic nervous system failed to inhibit the cold-induced increase in enzyme activities. The results suggest that the increase in enzymes is mediated by corticosteroids and not by either indolealkylamines or autonomic nervous system. The changes in the enzymes under cold stress with respect to the overshoot phenomenon, relationship to the degree of stress and reversibility on withdrawal from the stress indicate the "adaptate" nature of the response.

Differential Inhibition of Tryptophan Pyrrolase by Noradrenaline:

One puzzling observation in the time study shown above remains to be explained. The decrease in pyrrolase after reaching the peak is in striking contrast to the tyrosine aminotransferase. Both enzymes are known to be induced by cortisol, and in cold exposure the circulating corticosteroids continue to remain high during the entire experimental period of 48 hr. The specific decrease of pyrrolase notwithstanding high corticosteroids is shown to be due to noradrenaline which was found to inhibit the cortisol-mediated increase in pyrrolase, but not aminotransferase, at 3 hr. experimental period. This effect is specific to pyrrolase and to that induced by cortisol and not by tryptophan. Glucose, growth hormone and adrenaline inhibit

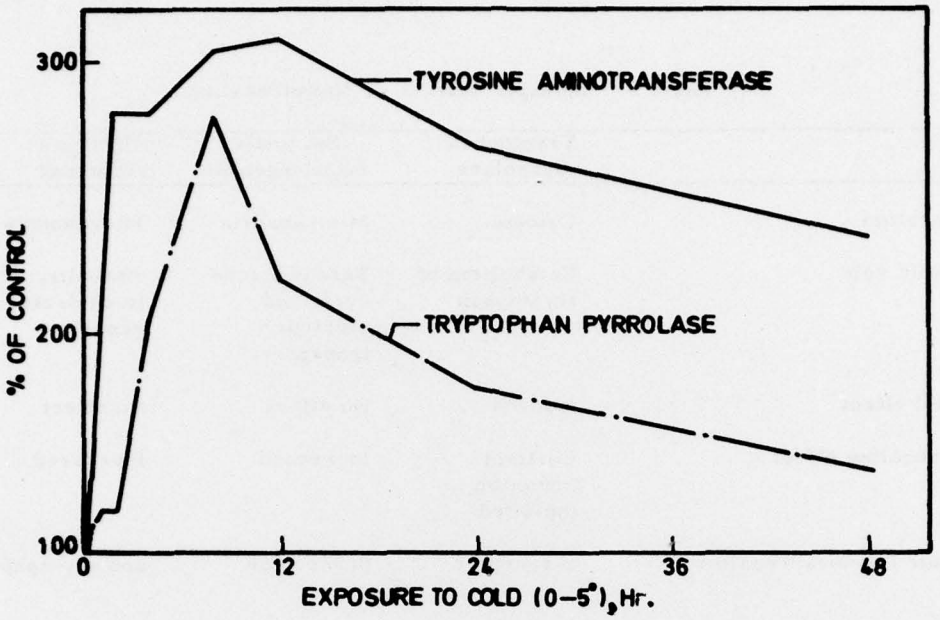


Fig. 6 Changes in catabolic enzymes of tyrosine and tryptophan in cold exposure

both enzymes. A large number of compounds and hormones tested were ineffective: serotonin, 5-hydroxytryptophan, dihydroxyphenylserine, FSH, LH, TSH, prolactin, glucagon, acetylcholine and colchicine. The noradrenaline effect is not by increasing endogenous competitive steroids. Only the α-adrenergic blocking agents, not the β-type, showed the capacity to reverse the inhibition and the α-agonists potentiated the effect of noradrenaline. The finding of mediation via α-receptors in the action of noradrenaline is interesting, yet it does not give any clue on how the inhibition is obtained. The β-receptor action is generally, associated with adenyl cyclase - cyclic AMP system. Participation of cyclic AMP in this case is ruled out. The events occurring after the interaction of noradrenaline with α-receptor and the stage at which the induction of this enzyme is specifically interfered remains to be elucidated.

Multiple effects of Noradrenaline: In this laboratory three systems have been studied for the effects of noradrenaline - the cytosolic enzyme tryptophan pyrrolase, the mitochondrial succinate dehydrogenase and the microsomal 3-hydroxy-3-methyl gultaryl (HMG)CoA reductase. The responses of these to the stress hormones are different as summarized in Table 3. The most striking information that emerged from these studies is that the unique cyclic AMP-mediation for hormone action is not the only mechanism and that noradrenaline, in some, yet unexplained fashion, functions within the cytosol to evoke its responses as in the case of cortisol.

Low pressure stress on aromatic amino acid catabolism: On exposure of rats to low atmospheric pressure the activities of tryosine aminotransferase and tryptophan pyrrolase increased after 9-12 hr. An environmental pressure decrease of about 0.5 atmosphere is needed for this change. Adrenalectomy completely abolished this change, as also treatment with inhibitors of protein systhesis. On continued exposure, phenylalanine hydroxylase increased by about 50% at 24 hr. Concomitant increase, in incorporation of labelled leucine into protein also occurred (Fig. 7). Both effects were abolished on treatment with cycloheximide at the beginning or during the hypobaric exposure showing thereby that proteins synthesized de novo in the early phase of the stress are needed for the increase of this enzyme at a later stage. The effect was traced to cortisol in view of the absence of the effect on young animals deprived of their pituitary-adrenal axis and also in adrenalectomized animals. It is confirmed that large doses of exogenous cortisol increased the enzyme, but 24 hr after the treatment and decreases thereafter. The effect is specific to corticosteroids. Further experiments showed that in regenerating liver after partial hepatectomy a decrease of this enzyme by about 50%

Table 3. Multiple Effects of Noradrenaline

	Tryptophan pyrrolase	Succinate dehydrogenase	HMGCoA reductase
Cell location	Cytosol	Mitochondria	Microsomes
Metabolic role	Catabolism of tryptophan	Part of Krebs cycle and electron transport	Anabolic, key step in cholesterol biogenesis
Cortisol effect	Induced	No effect	No effect
Noradrenaline effect	Cortisol induction inhibited	Increased	Increased
Receptor for noradrenaline effect	α-receptor	β-receptor	non α-, nonβ-
Dihydroxyphenyl serine (produces noradrenaline in cytosol)	No effect	Increased	Increased

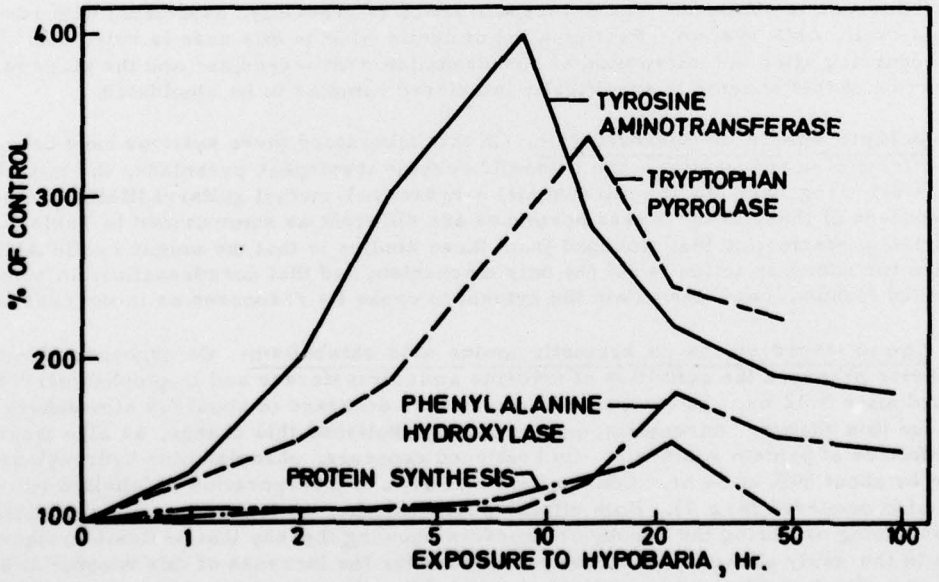


Fig. 7 Changes in enzymes of catabolism of aromatic amino acids on exposure to hypobaria

was found which could be reversed by treatment with cortisol but not with noradrenaline or growth hormone. These experiments provided novel examples of increase and decrease of phenylalanine hydroxylase in intact animals and it is hoped will lead to further understanding of the mechanism of modulation of this enzyme important in phenylketonuria.

MODIFICATIONS OF DRUG ACTION

Standardization of drug action is normally carried out at ambient conditions. As a result of drug action in combination with simultaneous stress conditions the body temperature may be changed and with it, the activities and stimulation of enzymes. A changed metabolism due to superposed environmental stress would alter the action of the drugs themselves. The use of protein synthesis inhibitors in animal experiments has become routine. The experiments described below show that caution is necessary in interpreting such results wherein additional variables of environment are involved.

Enzyme induction by a drug: Diethoxycarbonyldihydroxycollidine (DDC) is a drug well-known for its induction of δ-aminolevulinate (ALA) synthetase. While the stress conditions of hypobaria, hypoxia and cold did not alter this enzyme, its induction by DDC was inhibited when rats were simultaneously exposed to hypobaria or hypoxia but not cold. A period of 12-24 hr pre-exposure to hypobaria gave maximum inhibition which was reversed 12 hr after withdrawal to ambient pressure. The possible alteration of endogenous adenine nucleotides (but not cortisol) seem to be the cause, since treatment with ATP or cyclicAMP produced similar inhibition.

Drugs and body temperature under cold stress: Inhibitors of protein synthesis, notably cycloheximide, and colchicine, a microtubular disruptive agent, are hypothermic in intact rats exposed to cold. Agents that stimulate or inhibit preferentially the α-adrenergic receptors, are markedly hypothermic emphasising the recently discovered involvement of these receptors in thermogenesis. Among the compounds with marginal or little effect are: β-blocking agents, cholinergic agonists and antagonists, ouabain, inhibitors of L-aromatic amino acid decarboxylase, and ATP. Induction of tryptophan pyrrolase by cortisol correlated with body temperature under conditions of hypothermia as obtained above. Some preliminary experiments indicate that, under certain conditions, noradrenaline, serotonin or ATP can reverse the drug-induced hypothermia under cold exposure.

Reversal of lethal action of Actinomycin D: The use of actinomycin D as an inhibitor of transcriptional process has been well accepted. Most of the experiments reported in literature are usually done in short periods. Normally it is expected that the drug will be metabolized and removed and the inhibition released. This does happen with cycloheximide with restoration of protein synthesis by about 12 hr. It was of particular interest to observe that the group of actinomycin D-treated rats kept at ambient pressure showed signs of distress, moved very little and only 2/6 survived at 24 hr period. The group of actinomycin D-treated animals kept under hypobaric conditions behaved similar to the corresponding group without the drug, and all these animals survived the 24 hr. period of stress. Thus, hypobaric exposure prevented the mortality caused by the drug - a noteworthy observation of alteration of potency of a drug under environmental stress.

MODELS AND THEORIES

Interrelationship of food, nutrition and environment: In a seminar on "Science in the Service of Basic Human Needs" organised by ICSU Committee on Science and Technology for Developing Countries (COSTED), a model on this title had been presented, based on the experimental themes carried out in this project. It is instructive to note that the developing countries are approximately bounded by the north and south isotherms of 20°C. This region has hotter climate but accounts for poor productivity. Yields of crops should be higher but for the simultaneous increase of pests. Environmental temperature affects yields of milk and butterfat in cattle. Ironically population growth is high in these regions where every other productivity is low. The animal cell needs nutrition to provide energy and to supply essential compounds. These are used by a myriad of reactions to generate usable form of energy, ATP and releasing half the energy as heat. This reflects the efficiency of the energy transformations and is not

wasteful since the heat released is used to keep body temperature in homeotherms or endotherms. In cold exposure the heat is greater and is to be compensated by enhanced thermogenesis. It also leads to increased nitrogen loss and calls for higher protein. No information is available on this protein requirement in natural cold environment. On the other hand, in hot climate the calorie requirement is lowered and the nitrogen loss through sweat reaches significant proportion. The protein study group of FAO/WHO could not arrive at any conclusion on the protein needs under changed environmental temperature. On continued exposure the organism adapts to the imposed environmental stress by altering the whole panorama of metabolic networks with the ultimate aim of fitting the organism to the environment and nutrition. This is schematically represented in Fig. 8.

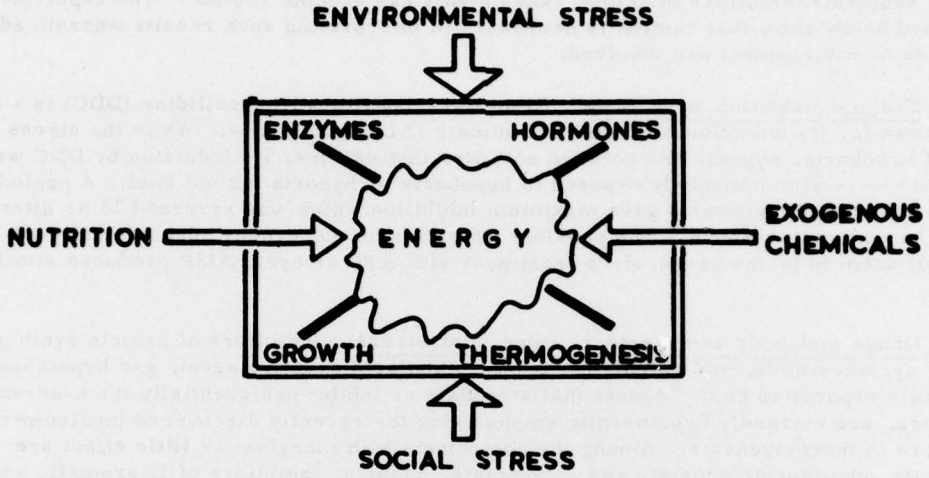


Fig. 8 Interrelationship of organism and environment

Hierarchical Modelling of Acclimatory Processes: The term acclimation has been used with several connotations in the field of acclimatory physiology. Acclimation can be defined as cumulative experience gained by the organism when subjected to a step change of a variable in the environment. Experimental observations showed that the changes obtained initially revert to basal and tend to stabilize at another steady state. Hierarchical systems theory of Mesarovic facilitates modelling of such complex system as in cold acclimation. In rats exposed to cold three major responses are observed - shivering and an increase in metabolic rats rate occur almost immediately; shivering progressively diminishes while metabolic rate continues to remain high due to non-shivering thermogenesis mediated via sympathetic nervous system; a decrease in metabolic rate at a late stage along with an increase in insulation to heat loss. The model proposes two major feedback loops. The environmental temperature and ethological inputs do disturb the thermoregulatory system which may be corrected by the control of heat generation subscribing to thermogenesis via the inner feedback loop. When the disturbances are large the outer feedback loop becomes operative and interacts suitably with the inner loop. In the hierarchical systems theory the infimal unit consists of the inner feedback loop and the heat generation and dissipation mechanisms. The supremal unit consists of the outer feedback loop becoming operational when the stress reaches a threshold value. Activation of specific neural pathways via the hypothalamus leads to neurohumoral signals as intervention inputs come from the supremal unit to the infimal unit. This model brings out the composite action within and between the various levels of organization in the process of acclimation to environmental changes.

Chemical Thermogenesis: In cold exposure the following features express themselves: increased demand of foodstuff, increased oxygen uptake, no irreversible uncoupling of oxidative phosphorylation, no damage to respiratory control, no increase in net ATP production, increased flux of electrons with energy being released as heat. Different tissues may respond differently and contribute to the overall heat budget. With respect to the liver the following

model is suggested, based on the properties of succinate dehydrogenase and its regulation by the redox status of ubiquinone.

1. Ubiquinone increases at the appropriate site of succinate dehydrogenase for activating the shunt pathway, represented by NT-reductase, and acts as a bypass of electrons.
2. A pool of ubiquinone in the membrane becomes reduced and this activates succinate dehydrogenase. Relative occupancy of the electron transport chain by succinate competes with and decreases oxidation of NADH.
3. Oxidation of NADH may then occur by the shunt pathway represented by NT-reductase. Additional NADH may also be transported out of mitochondria for oxidation.
4. Alternate pathways of tapping electrons from dehydrogenases or by transferring reducing equivalents to oxidases in peroxisomes or microsomes yield H_2O_2. Being cytotoxic H_2O_2 is destroyed by catalase present in mitochondria and peroxisomes. This being an exergonic reaction releases heat. In the model shown in Fig. 8, ubiquinone plays a central role by providing a shunt pathway for succinate dehydrogenase, by regulating the utilization of succinate and NADH and acting as a pool for transferring reducing power to other particulates. In this process, ubiquinone may act as a switch. It is conjectured that ubiquinol-H_2O_2-catalase system may turn out to be an universal mechanism for all the thermogenic cells (Fig. 9).

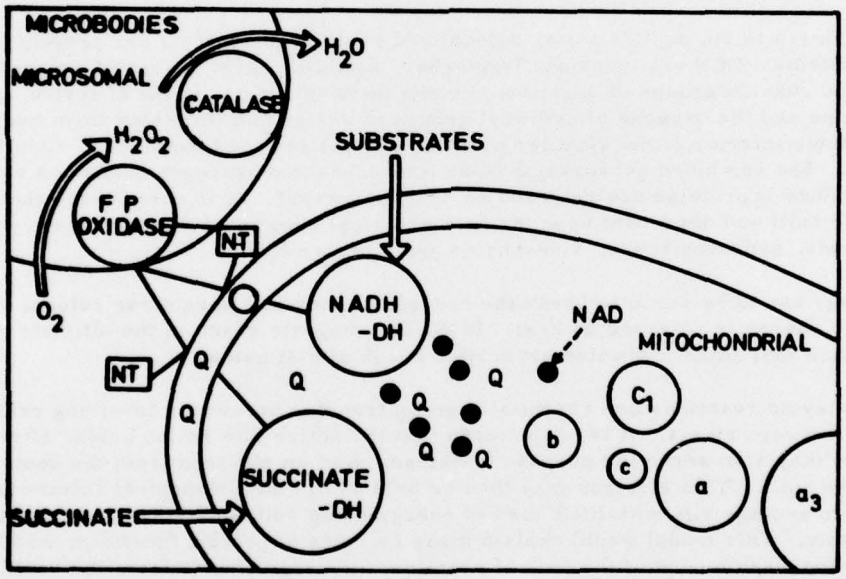


Fig. 9 A Model for Chemical Thermogenesis

π-H Pathways and functions of Proteins: A close examination of α-helix reveals that the intra-chain hydrogen bonds themselves form interesting repeating sequences superimposed on the peptide helix. Each peptide group in the helix can be viewed as being held between two α-carbons and linked to another through a hydrogen bond. This structural feature provides a continuous helical sequence of alternating peptide groups and hydrogen bonds (..HN..C..O..HN..C..O..) with the sense of winding opposite that of peptide helix. This intrinsic structural feature is called "suprahelix" (Fig. 10).

In the theoretical calculations of delocalization of π-electrons of peptide bond across a hydrogen bond, the energy gap of about 3 eV or higher between the highest filled and lowest empty bands was obtained. It is now clear that delocalization of π-electron in the suprahelix

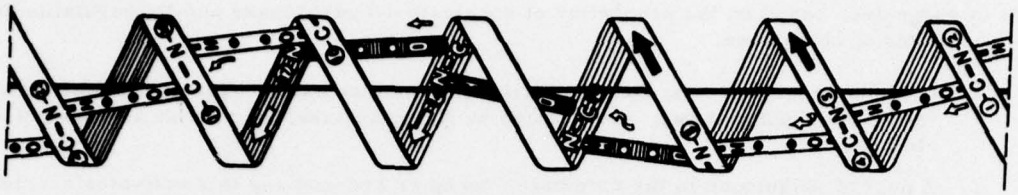


Fig. 10 Suprahelix : Schematic diagram showing one of the three suprahelical sequences of hydrogen bonds winding in the opposite sense of α-helix (N = 6, D = 4.5Å, P = 27 Å).

described above is not possible. In other words, semiconduction in polypeptide structure seems remote. It is instructive to note the life cycle of a migrating electron in biological systems - it originates from oxygen of water during photolysis in chloroplasts, travels through a variety of cell components and structures and returns to oxygen during respiration in mitochondria. The electron, therefore, is extraneous to the protein structure. Using an extraneous electron, calculations of molecular orbitals showed that the entering electron is delocalized in the hydrogen-bonded peptide units with individuality of each unit being lost. Derivative to this is the possibility of electronic conductivities being higher in sections than the whole protein as now measured. Should this prove feasible, an extraneous electron can migrate within sections of polypeptide structures where such interlinked π-electron systems and hydrogen bonds exist.

In addition to the peptide units, delocalized π-electron systems are present in some side chains of proteins. Of these tyrosine, tryptophan, histidine, acid groups of asparagine and glutamine and guanido groups of arginine, the nitrogens of amino groups of lysine, glutamine and asparagine and the oxygens of hydroxyl groups of serine and threonine form hydrogen bonds. The hydrophobic interior of the globular proteins protect and aid formation of significant hydrogen bonding. The combined networks of these interconnected hydrogen bonds and π-electron systems obtained in proteins are referred as "π-H pathways". It is coincidence that the π-H pathways are built and dependent upon the four principal structural features of enzyme proteins - peptide bonds, hydrogen bonds, side-chains and globular fold.

Energy has to be put in to break the hydrogen bonds and when these reform the native structure the energy is released as heat. In the thermogenic reaction the ultimate process of heat production may involve similar reversible break of π-H pathways.

All enzyme reactions are essentially group transfer processes involving redistribution of electrons between atoms. It is conjectured that the active site amino acids, after binding to the substrate may also serve the purpose of extraction of an electron from the donor atom, of the bond to be split. This electron may then be held within the polypeptide framework and be transferred to another site with little loss of energy using suitable π-H pathways characteristic of each enzyme. This model would explain many features of protein functions, and foremost of all the indispensable need of the bulk of proteins. Hydrogen bonds form the basic foundations of life process, in water structure, and in keeping fidelity, storage, and transfer of genetic information. This innate structural feature of hydrogen bonding may indeed become vital in protein functions.

SYMPOSIA/SEMINARS

	Title of the talk	Date	Symposium/University
1.	Tryptophan metabolism under hypobaric conditions.	Sept. 21, 1971	University of Liverpool, Liverpool, U. K.
2.	Succinate oxidation under hypobaric conditions.	Sept. 22, 1971	University of Warwick, Coventry, U. K.
3.	Modification of succinate dehydrogenase under hypobaric and hypoxic conditions.	Dec. 1, 1971	"Symposium on Environmental Biochemistry" Golden Jubilee meetings of the Department of Biochemistry, I. I. Sc., Bangalore.
4.	Research on Environmental Stress	Jan. 3, 1972	Central Public Health Engineering Research Institute (CSIR), Nagpur.
5.	Environmental Biochemistry	Feb. 12, 1972	"Symposium on Biology", Biological Society of Trichy, Trichy.
6.	Life processes under extreme environmental conditions	May 4, 1972	Indian Institute of World Culture, Bangalore
7.	Modification of succinate dehydrogenase under environmental stress	June 27, 1972	Babha Atomic Research Centre, Bombay
8.	Modification of succinate dehydrogenase under environmental stress	Oct. 11, 1972	V. P. Chest Institute, University of Delhi, Delhi
9.	Hypoxic activation of succinate dehydrogenase	Oct. 12, 1972	All India Institute of Medical Sciences, New Delhi
10.	Decrease in the induction of δ-aminolevulinate synthetase under hypobaria	Nov. 20, 1972	Society of Biological Chemists, Annual Meetings, Pantnagar
11.	Modification of succinate dehydrogenase under hypoxia..	Nov. 24, 1972	Society of Biological Chemists, Madras
12.	Modification of succinate dehydrogenase under environmental stress	Dec. 18, 1972	Punjab University, Chandigarh
13.	Tryptophan metabolism under hypobaria	Dec. 19, 1972	Punjab University, Chandigarh
14.	Multiple control of cellular oxidation by ubiquinone	Feb. 12, 1973	Symposium on "Control Mechanisms in Cellular Processes", BARC, Bombay
15.	Activation of succinate dehydrogenase	April 10, 1973	Biochemical Society Symposium, I. I. Sc., Bangalore
16.	Environmental Stress and Biochemical Adaptation	June 29, 1973	Summer Institute in Biosciences, I. I. Sc., Bangalore
17.	Modification of succinate dehydrogenase in response to environmental stress conditions of hypobaria and hypoxia	July 7, 1973	Biochemical Society Symposium, I. I. Sc., Bangalore
18.	Mechanism of induction of tyrosine aminotransferase in cold	July 7, 1973	Biochemical Society Symposium, I. I. Sc., Bangalore
19.	Enzyme modification in adaptation (theme talk)	July 7, 1973	Biochemical Society Symposium, I. I. Sc., Bangalore
20.	A molecular mechanism of thermogenesis	Aug. 20, 1973	Osmania University, Hyderabad
21.	Activation constant for environmental stress	Oct. 13, 1973	Biochemical Society Symposium, I. I. Sc., Bangalore
22.	Mechanism of induction of tryptophan pyrrolase in cold exposure	Nov. 1, 1973	Society of Biological Chemists (India), Annual Meetings, Mysore
23.	Regulation of thermogeneis by modifications of succinate dehydrogenase	Dec. 13, 1973	International Symposium on Biomembranes, Madurai
24.	Regulation of succinate dehydrogenase	Feb. 10, 1974	Guha Research Conference, Mahabaleshwar
25.	Environmental Stress and Biochemical Adapatations (2 lectures)	Feb. 25/26, 1974	S. N. Medical College, Agra
26.	Suprahelix	Mar. 5, 1974	Molecular Biophysics Unit, I. I. Sc., Bangalore
27.	Biological Rhythms	Aug. 30, 1974	The Biological Society, Tiruchirapalli
28.	Modulation of steroidmediated induction of hepatic enzymes by noradrenergic nervous system in cold exposure	Oct. 20-26, 1974	International Congress of Physiology, New Delhi
29.	Enzymes of tyrosine metabolism under conditions of environmental stress	Nov. 1-3, 1974	Society of Biological Chemists (India), Annual Meetings, Ludhiana
30.	Hierarchical regulation of tryptophan metabolism in cold exposure	Nov. 1-3, 1974	Society of Biological Chemists (India), Annual Meetings, Ludhiana
31.	Multiple hormonal regulation of rat liver tryptophan 2, 3-dioxygenase	Dec. 13, 1974	Biochemical Society Symposium, I. I. Sc., Bangalore
32.	A multichannel telethermometer for physiological studies of thermoregulation in experimental animals	Jan. 31-Feb. 1, 1975	All India Seminar on "Technological Progress of Instrumentation", Madras
33.	Response of tyrosine aminotransferase and phenylalanine in liver of rats exposed to hypobaric hypoxia	Feb. 15, 1975	Biochemical Society Symposium, I. I. Sc., Bangalore
34.	Electron transport in polypeptide structures	Feb. 26, 1975	Guha Research Conference, Mysore

Symposia/Seminars (contd.)

	Title of the talk	Date	Symposium/University
35.	Protein synthesis in adaptation to stress	Mar. 2-8, 1975	Symposium on genetics and development, Mahabaleshwar
36.	Hierarchical basis of acclimation processes - a mathematical model for acclimatory	May 1975	Symposium on Mathematical Models in Biology, Bandipur National Park
37.	Hypobaric stress and enzymes in the metabolism of tyrosine	July 27-30, 1975	International Symposium on "Catecholamines and Stress", Bratislava, Czechoslovakia
38.	Information processing in living cells	Aug. 8-9, 1975	Biochemical Society Symposium, I. I. Sc., Bangalore
39.	Biocatalysis - Unique design of enzyme proteins	Nov. 8, 1975	Symposium on catalysis, Indian Academy of Sciences, Nagpur
40.	Enzyme changes and nutritional requirements under environmental stress	Dec. 8-10, 1975	Symposium on "Nutrition under environmental stress", Defence Food Research Laboratory, Mysore
41.	A novel theory on catalysis by proteins	Jan. 9, 1976	50th Biochemical Society Symposium, I. I. Sc., Bangalore
42.	Secondary structure of enzyme proteins	Mar. 9, 1976	Guha Research Conference, Chorwad, Gujarat
43.	Molecular basis of thermogenesis	July 13, 1976	Symposium on Bioenergetics, Goa
44.	Energy transformations in biosystems (Theme talk)	July 13, 1976	Symposium on Biogenetics, Goa
45.	Inter-relationship of food, nutrition and environment	Aug. 11, 1976	A COSTED Seminar on "Science in the service of basic human needs", Hyderabad
46.	Effect of environmental stress of cold and hypobaria on Coenzyme Q metabolism and Coenzyme Q dependent Reactions	Sept. 16, 1976	International Symposium on Coenzyme Q, Lake Yamanaka, Japan
47.	A theory of catalysis by proteins	Sept. 22, 1976	University of Toyama, Toyama, Japan
48.	Regulation of phenylalanine hydroxylase	Sept. 24, 1976	University of Kyoto, Kyoto, Japan
49.	Food, nutrition and environment	Nov. 4, 1976	ASHA-COSTED seminar on Science and Human Development, Bangalore
50.	A model for chemical thermogenesis regulated by ubiquinone	Dec. 11, 1976	Symposium on "Vitamin and carrier functions of polyprenoids", Bangalore
51.	Thermogenesis	Dec. 17, 1976	University of Allahabad, Allahabad
52.	Adaptation to stress	Dec. 20, 1976	Banaras Hindu University, Varanasi
53.	Modulation of succinate dehydrogenase	Dec. 28, 1976	Indian Institute of Experimental Medicine, Calcutta
54.	Theory of protein catalysis	Dec. 30, 1976	Bose Institute, Calcutta
55.	Modulation of succinate dehydrogenase	Jan. 19, 1977	J. N. University, Delhi
56.	Secondary structure in protein functions	Jan. 20, 1977	J. N. University, Delhi
57.	A theory on protein catalysis	Jan. 21, 1977	Delhi University, Delhi
58.	Multiple effects of noradrenaline on hepatic enzymes	Mar. 1, 1977	International seminar on "Stress in Health and Disease", Varanasi

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28. Activation of succinate dehydrogenase in brown adipose tissue mitochondria
S. Sivaramakrishnan and T. Ramasarma

29. Oxidation of succinate in noradrenaline-treated rats
S. Sivaramakrishnan and T. Ramasarma

30. Activation of succinate dehydrogenase in isolated mitochondria by noradrenaline
S. Sivaramakrishnan and T. Ramasarma

31. *Regulation of hepatic phenylalanine hydroxylase under conditions of* hypobaria, cortisol treatment and regeneration
M. A. A. Namboodiri, R. Manjunath, S. P. Bhat and T. Ramasarma

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Ph. D. THESES ARISING OUT OF THE WORK

1. L. Susheela: Activation of succinate oxidation in response to environmental stress

2. M. A. A. Namboodiri: Metabolism of tyrosine under conditions of environmental stress

3. V. Sitaramam: Metabolism of hepatic tryptophan pyrrolase under conditions of environmental stress

4. S. Sivaramakrishnan: *Modulation of* succinate dehydrogenase under conditions of environmental stress and treatment with noradrenaline

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ENVIRONMENTAL CHAMBERS