Biochimica et Biophysica Acta, 321 (1973) 423–436

BBA 67036

MODULATION OF SUCCINATE DEHYDROGENASE IN RESPONSE TO ENVIRONMENTAL STRESS CONDITIONS OF HYPOBARIA AND HYPOXIA

L. SUSHEELA AND T. RAMASARMA

Department of Biochemistry, Indian Institute of Science, Bangalore 560012 (India)

(Received May 16th, 1973)

SUMMARY

1. An increase in the oxidation of succinate by hepatic mitochondria in rats exposed to hypoxia (O_2–N_2; 1:9, v/v) or hypobaria (0.5 atm) was observed which appears to be due to modification of the activity of the rate-limiting succinate dehydrogenase [succinate: (acceptor) oxidoreductase, EC 1.3.99.1].

2. The qualitative nature of this increase was indicated by obtaining the same maximal activity in both control and the exposed groups on activation by preincubation of such mitochondria with succinate and also by the failure to prevent the changes on treatment of the animals during the exposure to stress with the protein-synthesis inhibitor cycloheximide.

3. The increase in enzyme activity was progressive with the time of exposure and reached a maximum by 4 h and was maintained at this high level under hypoxia for 36 h but reverted to the basal level under hypobaria by 12 h. On withdrawal of the stress at 4 h the activity reverted to the basal level in 6 h after hypobaric stress and in 12 h in hypoxic stress.

4. The increased activity was stable to repeated washing *in vitro* of such mitochondria with the sucrose homogenizing medium. A similar washing procedure after preincubation with succinate, which may displace a firmly bound effector, however, reversed the hypoxic activation but not the hypobaric activation.

5. Although succinate:neotetrazolium reductase measures the same flavoprotein, activity assayed by this method showed no changes except for the significant increase at long exposure of 36 h only under hypoxia.

6. The results suggest that the modulation of the succinate dehydrogenase activity in hypobaria and hypoxia appears to be a compensatory mechanism invoked to overcome the effect of lowered O_2 tension prevalent in both the stress conditions.

INTRODUCTION

Animals exposed to hypobaria and hypoxia develop the capacity to overcome

Abbreviations: NT, 2,2′,5,5′-tetraphenyl-3,3′-(4,4′-biphenylene)-ditetrazolium chloride; PMS, *N*-methylphenazoniummethyl sulphate (phenazine methosulphate).

the deleterious effects of these stresses by invoking compensatory reactions whereby the vital energy requirements could be maintained. Apart from the lowered O_2 tension which tends to decrease the availability of O_2 to the tissues, animals exposed to hypobaria are subjected to an additional stress of lowered atmospheric pressure. While the physiological effects obtained under hypobaria have often been ascribed to the hypoxia prevalent under this condition, practically nothing is known about the adaptation processes to lowered atmospheric pressure itself.

It was first reported from our laboratory that the hepatic mitochondrial succinate dehydrogenase [succinate:(acceptor) oxidoreductase, EC 1.3.99.1] was raised from the basal level to a partially activated state on brief exposure of rats to hypobaria[1]. This increase in the activity of succinate dehydrogenase was also observed when rats were exposed for short periods to a low partial pressure of O_2, equivalent to that of the hypobaric condition[2] and hence appeared to be an effect of lowered O_2 tension. Nevertheless, hypobaria *per se* has been shown to elicit a specific increase in the activities of hepatic tryptophan pyrrolase and tyrosine aminotransferase on exposure of rats even for short periods of time[2]. This response in the enzyme activities was not observed in exposures to hypoxia.

In view of the differential alteration of enzyme activities in response to hypobaria and hypoxia, it was of interest to carry out a comparative study of the specific responses elicited in the energy metabolism with respect to the oxidation of succinate in these two conditions of stress. The results presented in this paper show that the modulation of the rate-limiting step of the succinate oxidase system, although by different effectors in hypobaria and hypoxia, appears to be a compensatory mechanism to overcome the effects of the low partial pressure of O_2 prevalent in both the conditions.

EXPERIMENTAL

Phenazine methosulphate (PMS) was purchased from Calbiochem, Inc. Los Angeles, Calif. (U.S.A.) and cytochrome *c* from Sigma Chemical Co., St. Louis, Mo. (U.S.A.). All the other chemicals used were of the Analar grade and were dissolved in water double-distilled in an all-glass apparatus.

Animals and exposure to hypobaria and hypoxia

Male albino rats weighing 150–170 g, from the stock colony of the Institute were used. For hypobaric exposure, rats were exposed to an atmospheric pressure of 350 ± 5 mm Hg (corresponding to an altitude of about 6100 m) in a decompression chamber. For exposure to hypoxia, rats were exposed to a gas mixture composed of O_2–N_2 (1:9, v/v) which gave a partial pressure of O_2 equivalent to that in the hypobaric conditions. A detailed description of the experimental chambers is given in a previous paper[3]. At the end of the experimental period atmospheric pressure was restored in the chambers. The animals were removed and killed along with the unexposed controls, by stunning and decapitation. The livers were removed, chilled and processed further.

Preparation of rat liver mitochondria

The method followed for the isolation of rat liver mitochondria has been

described elsewhere[4]. Protein was determined by the biuret method after solubilization with deoxycholate.

Enzyme assays

Succinate dehydrogenase. Succinate dehydrogenase activity was determined spectrophotometrically with PMS and 2,6-dichlorophenolindophenol as the electron acceptor system as described by Arrigoni and Singer[5]. The enzyme activity was assayed at 25 °C and one unit of activity equals 1 μmole of dye reduced/min per mg protein.

Succinate:neotetrazolium reductase. Succinate:neotetrazolium reductase activity in mitochondria was determined by measuring the formazan produced on enzymic reduction of 2,2′,5,5′-tetraphenyl-3,3′-(4,4′-biphenylene)-ditetrazolium chloride (NT) (ref. 6). The details of the method of assay are described elsewhere[3]. One unit of enzyme activity represents 1 μmole of formazan formed/min per mg protein.

Succinate oxidase. The O_2 uptake was measured polarographically with the Gilson KM Oxygraph. The reaction medium contained 100 μmoles of potassium phosphate buffer (pH 7.4), 167 μmoles of potassium succinate (pH 7.4), 33 nmoles of cytochrome *c*, 1.3 μmoles each of $AlCl_3$ and $CaCl_2$ and 1–2 mg of mitochondrial protein in a total volume of 2 ml. The uncoupled succinate oxidation was measured by determining the O_2 uptake at 25 °C. One unit of enzyme activity equals 1 μatom of O_2 used/min per mg protein.

Cytochrome oxidase. Cytochrome oxidase (EC 1.9.3.1, cytochrome *c*:O_2 oxidoreductase) activity was determined manometrically[7]. The reaction mixture contained 100 μmoles of potassium phosphate buffer (pH 7.4), 0.25 μmoles of cytochrome *c*, 1.3 μmoles of $AlCl_3$, 34.2 μmoles of ascorbate (neutralized) and 0.2–0.5 mg of mitochondrial protein in a total volume of 3.0 ml. The O_2 uptake was determined after an initial 5 min equilibration at 30 °C. The activity is expressed as units/g protein where a unit of activity corresponds to 1 μatom of O_2 utilised per min.

Succinate:cytochrome c reductase. The enzyme activity was assayed spectrophotometrically as described by Tisdale[8]. The reaction mixture contained 30 μmoles of potassium phosphate buffer (pH 7.4), 30 μmoles of KCN, 0.6 μmole of EDTA, 5 mg of bovine serum albumin, 30 μmoles of potassium succinate (neutralized) and 130–150 μg of mitochondrial protein in a total volume of 2.9 ml. The reaction mixture was incubated at 37 °C for 2 min and the reaction was started by adding 1 mg of ferricytochrome *c* (0.1 ml) and the change in absorbance was measured at 550 nm against a control lacking only the enzyme. The change in absorbance at 550 nm was followed at 10-s intervals over the first 2 min in a Beckmann DB Recording Spectrophotometer. Using the extinction coefficient for cytochrome *c* (reduced–oxidized) as $2.1 \cdot 10^4$ cm²/mmole, the specific activity was calculated as $\Delta A_{550\ nm}$/min per mg protein/21. A unit of enzyme activity represents 1 μmole of cytochrome *c* reduced/min per mg protein.

Preincubation of mitochondria for activation

Preincubations of mitochondria were carried out at 37 °C for 7 min[9]. The preincubation mixture contained about 2 mg of mitochondrial protein, 100 μmoles of potassium phosphate buffer (pH 7.6) and 50 μmoles of potassium succinate in a

total volume of 1 ml. An aliquot of this mixture was used for assaying the enzyme activity.

Washing the activated mitochondrial preparations

The procedure followed for washing the activated mitochondrial samples was the same as described previously[4]. In these experiments about 25–40 mg of the mitochondrial protein were used.

In all the experiments, six rats were used in each group unless otherwise stated. Since appreciable variations in the enzyme activities were encountered within the animals killed on different days, simultaneous controls were run, for every time interval. However, within the group killed on the same day, the difference between the controls and the experimental groups were consistent and reproducible. Statistical analyses of the data were carried out by the Student *t* test.

RESULTS

Activation of succinate dehydrogenase during brief exposure to hypobaria and hypoxia

Exposure of rats to hypobaria or hypoxia for a short period of 4 h resulted in a significant increase of the activity of succinate oxidase (Table I). This increase was not due to an overall enhancement of the activity of the whole respiratory system and appeared to be due to a specific increase in the activity of succinate dehydrogenase, the rate-limiting step in the oxidation of succinate. This was further substantiated by the increase observed in the activity of succinate:cytochrome *c* reductase which includes the rate-limiting step catalyzed by the primary dehydrogenase, and the absence of any significant stimulation in the activity of cytochrome oxidase which is known to be far in excess of the overall rate of oxidation of succinate.

It is interesting to note from the data in Table I that unlike the succinate

TABLE I

CHANGES IN THE ACTIVITIES OF THE MITOCHONDRIAL SUCCINATE OXIDASE SYSTEM ON EXPOSURE TO HYPOBARIA AND HYPOXIA

Rats were exposed to hypobaria or hypoxia for 4 h. At the end of this period, the animals were restored to ambient pressure, killed and the livers were processed for the separation of the mitochondrial fraction. The enzyme activities were determined as described in the text. The values represent the means ± S.D. of samples processed individually from 8 animals in each group. The figures in parentheses indicate the % control activity. The values given for succinate dehydrogenase activity represent the basal enzyme activity assayed without preincubation with succinate.

Enzyme activity	*Units/g protein*		
	Control	*Hypobaria*	*Hypoxia*
Succinate oxidase	102 ± 19	*159 ± 28 (156)	*152 ± 26 (149)
Succinate:cytochrome *c* reductase	61 ± 11	* 88 ± 10 (144)	*114 ± 10 (187)
Cytochrome oxidase	550 ± 100	553 ± 125 (101)	578 ± 74 (105)
Succinate dehydrogenase	44 ± 13	* 75 ± 16 (170)	* 79 ± 20 (179)
Succinate:NT reductase	11.4 ± 1.6	12.4 ± 3.2 (109)	12.2 ± 1.9 (107)

* Controls *vs* exposed, $P < 0.01$.

dehydrogenase, the succinate:NT reductase activity which is also catalyzed by the same flavoprotein as the dehydrogenase, did not increase in both the stress conditions. This could presumably be due to a differential alteration of the dye acceptor sites of the enzyme protein at the molecular level in hypobaric and hypoxic conditions.

A time-course of the activation of succinate dehydrogenase in the animals exposed to hypobaric and hypoxic stress is shown in Fig. 1. A significant increase (35–40%) in the basal enzyme activity could be discerned as early as 2 h in the animals exposed to either hypobaria or hypoxia. A maximum stimulation in the enzyme activity was reached at 4 h of exposure to either of the stress conditions. However, on longer exposures to the two stress conditions, a differential response in the enzyme activity was observed. In hypobaria, there was a progressive decline in the enzyme activity beyond 4 h, reaching the basal levels by 12 h. On the other hand, the stimulation observed in hypoxia was sustained at the elevated levels even after 36 h.

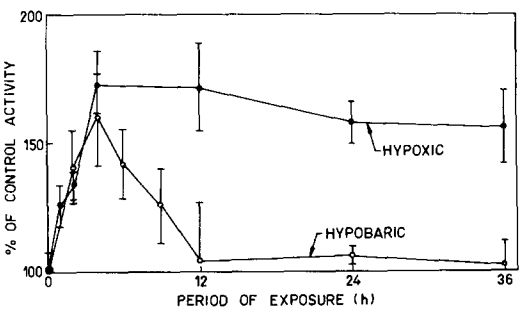

Fig. 1. Effect of exposure to hypobaria and hypoxia on the activities of succinate dehydrogenase in rat liver. Animals were exposed to hypoxia or hypobaria for the time indicated and the hepatic mitochondrial succinate dehydrogenase activity (basal) was determined without preincubation with succinate. The details of the assay are given in the text. The points indicate the mean ± S.D. of samples processed individually from 8 animals in each group. P values, calculated by the t test, were <0.01 at every point when compared to the unexposed controls.

Effect of hypobaric and hypoxic exposures on the succinate-stimulated enzyme activity

Studies by Kearney[10] and Kimura *et al.*[9] have shown that preincubation of mitochondria with succinate or substrate competitors like malonate, stimulated the succinate dehydrogenase activity. A 2-fold stimulation could be observed with rat liver mitochondria[1]. The binding of the effectors to the enzyme protein appeared to produce a conformational change resulting in increased catalytic activity[10]. In view of this known activation phenomenon, it was of interest to study whether the activation observed under hypobaric and hypoxic exposures was of a similar nature.

The partially activated mitochondrial samples obtained from livers of animals exposed to hypobaria and hypoxia were preincubated with succinate at 37 °C for 7 min and the enzyme activities assayed after preincubation. In hypobaric exposures, the basal enzyme activity was observed to increase progressively up to 4 h and then decline beyond this period. Assayed after preincubation of such mitochondria with succinate, the same maximum activity was reached as those of the unexposed controls in all the time periods tested (Table II). A similar pattern of the succinate-stimulated

TABLE II

EFFECT OF EXPOSURE OF ANIMALS TO HYPOBARIA AND HYPOXIA ON THE HEPATIC MITOCHONDRIAL SUCCINATE DEHYDROGENASE ACTIVITY

Animals were exposed to hypobaria or hypoxia for the periods indicated and the enzyme activities were determined with and without preincubation with succinate. The details of the assay are as mentioned in the text. The values represent the means ± S.D. of samples processed individually from 8 animals in each group. Due to variations encountered in the enzyme activities in the control animals, separate controls without the exposures were run at each time interval.

Period of exposure (h)	*Units/g protein*			
	Control		*Exposed*	
	Basal activity	*Activated with succinate*	*Basal activity*	*Activated with succinate*
I. Exposed to hypobaria				
2	58 ± 1	92 ± 4	82 ± 12*	113 ± 11
4	40 ± 3	127 ± 9	64 ± 12*	123 ± 11
6	48 ± 9	118 ± 9	68 ± 9*	94 ± 3
9	48 ± 9	118 ± 18	61 ± 10*	108 ± 10
12	47 ± 11	122 ± 18	49 ± 17	125 ± 25
24	56 ± 5	149 ± 15	60 ± 2	152 ± 14
36	52 ± 9	169 ± 23	53 ± 7	165 ± 20
II. Exposed to hypoxia				
1	47 ± 4	93 ± 10	59 ± 5*	94 ± 10
2	47 ± 3	93 ± 10	63 ± 4*	92 ± 11
4	40 ± 3	127 ± 9	69 ± 9*	138 ± 14
12	47 ± 11	122 ± 18	81 ± 14*	140 ± 35
24	45 ± 6	96 ± 9	71 ± 6*	116 ± 23
36	57 ± 4	97 ± 10	89 ± 14*	105 ± 5

* Basal activity, controls *vs* exposed, $P < 0.01$.

enzyme activity was also observed in the animals exposed to hypoxia. While the basal enzyme activity showed a progressive increase up to 4 h and was sustained at the elevated level for 36 h, the maximal activity reached after activation with succinate was the same as those of the controls (Table II). This suggested that the activation observed in conditions of hypobaria and hypoxia was the result of a qualitative change at the molecular level, since a quantitative change in the enzyme protein should have given a higher total specific activity for the maximal activity as compared to that of the unexposed controls.

Pretreatment with cycloheximide

The qualitative nature of the change was further substantiated by the results obtained on treating the animals with the inhibitor of protein synthesis, cycloheximide, prior to the exposures. Only one time interval was chosen for the exposure, this period being sufficient to elicit the maximal stimulation of the enzyme activity in both the stress conditions. The results presented in Table III show that treatment of the animals with cycloheximide prior to the exposures did not prevent the increase in the basal activity of succinate dehydrogenase, observed during the exposure to hypoxia. Such an observation has also been made in animals exposed to hypobaria[1]. However, neither the exposure to hypoxia nor the treatment with cycloheximide had any effect on the succinate-stimulated enzyme activity. These observations support

TABLE III

EFFECT OF CYCLOHEXIMIDE TREATMENT ON THE SUCCINATE DEHYDROGENASE ACTIVITY IN RATS EXPOSED TO HYPOXIA

Cycloheximide (250 μg/rat) was injected intraperitoneally into rats, divided into two groups of 6 animals in each group. After 30 min, one group was exposed to hypoxia for 4 h. The activity of hepatic mitochondrial succinate dehydrogenase was determined with and without preincubation with succinate. The values presented are means ± S.D. of samples processed individually from 6 animals in each group.

Treatment	*Units/g protein*			
	Control		*Hypoxia*	
	Basal activity	*Activated with succinate*	*Basal activity*	*Activated with succinate*
None	40 ± 3	127 ± 9	69 ± 9*	138 ± 14
Cycloheximide	50 ± 3	111 ± 14	75 ± 6*	120 ± 9

* Controls *vs* hypoxia, $P < 0.01$.

the conclusion that the increased succinate dehydrogenase activity in the stress conditions is not due to protein synthesis *de novo*, but to enhanced specific activity of the preformed enzyme. The use of cycloheximide as the inhibitor for protein synthesis in these studies concerned with a mitochondrial enzyme, is justified in view of the overwhelming evidences in favour of the synthesis of mitochondrial enzymes at the ribosomal sites[11].

Effect of varying concentrations of succinate

Kinetic studies on the effect of succinate concentration on the activity of the enzyme were next made. The enzyme activities in the mitochondrial samples obtained from both the controls and the animals exposed to hypoxia gave a similar response to increasing substrate concentrations, in either basal or activated states. Lineweaver-Burk plots shown in Fig. 2 indicate that the lines are nearly parallel with both K_m and V increasing. The increased K_m values showed that the concentration of succinate required for saturation increased for the activated enzyme thus indicating the possibility of a change in the affinity for the substrate (Table IV).

Stability of the activated preparations to washing with the sucrose homogenizing medium

Succinate dehydrogenase is known to be activated by a variety of compounds like dicarboxylates, ubiquinols, nitrophenols and pyrophosphates[3]. Kimura *et al.*[9] have shown that activation by succinate differed from that of the competitive inhibitors in being reversed by repeated washing of the activated mitochondrial preparations with the sucrose homogenizing medium. On the other hand, the enhanced enzyme activity in the mitochondrial preparations activated by an effector like malonate, which is known to bind firmly to the enzyme protein, could not be reversed to the basal level by such a simple washing procedure. Hence, the stability to such a washing procedure gives an insight into the nature of the activator bound to the enzyme protein.

Using this diagnostic approach the effect of washing on the reversibility of the activations observed under hypobaric and hypoxic stress was studied. The results

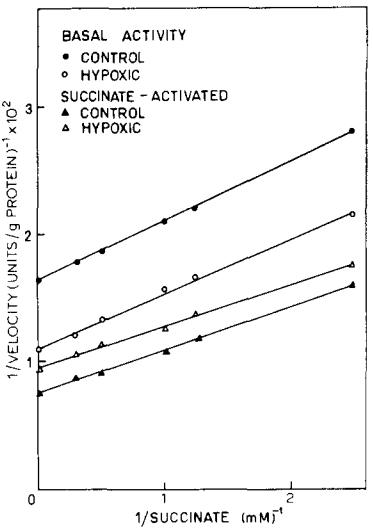

Fig. 2. Effect of succinate concentration on the activity of succinate dehydrogenase in the liver of rats exposed to hypoxia. The enzyme activities were determined with and without preincubation with succinate, at various concentrations of succinate added in the assay. The values at each point represent the means of the analyses of samples processed individually from 2 animals in each group In this experiment the data obtained were treated by the method of least squares to obtain the lines shown in the figure with points showing the experimental values.

TABLE IV

APPARENT K_m VALUES FOR SUCCINATE DETERMINED FOR SUCCINATE DEHYDROGENASE FROM NORMAL AND HYPOXIC-ACTIVATED MITOCHONDRIA

Enzyme activities were determined with and without preincubation with succinate as described in the text.

Succinate dehydrogenase	*K_m succinate (M)*
I. Basal activity	
Normal	$2.8 \cdot 10^{-4}$
Hypoxia	$3.9 \cdot 10^{-4}$
II. Succinate-activated	
Normal	$4.5 \cdot 10^{-4}$
Hypoxia	$3.3 \cdot 10^{-4}$

obtained were compared with a similar treatment carried out with two activated mitochondrial preparations obtained by preincubating mitochondria with ubiquinol-9 and oxaloacetate.

The results in Table V suggest that both ubiquinol-9 and oxaloacetate (well-known activators of succinate dehydrogenase) stimulate the enzyme activity 2–3-fold which was stable to washing with the sucrose homogenizing medium. Nevertheless, a second preincubation with succinate, followed by repeated washings with the medium reversed the enzyme activity to the basal level in the case of oxaloacetate activation. In the case of ubiquinol-9, the enzyme activity still remained at the partially activated state.

A similar treatment carried out on the partially activated mitochondrial

TABLE V

A COMPARISON OF THE EFFECTS OF WASHING ON SUCCINATE DEHYDROGENASE ACTIVITY IN THE PREPARATIONS ACTIVATED BY UBIQUINOL, OXALOACETATE, HYPOBARIA AND HYPOXIA

About 25 mg of mitochondrial protein were preincubated at 37 °C with 100 μmoles of potassium phosphate buffer (pH 7.4), ubiquinol-9 (0.5 mM) or oxaloacetate (0.077 mM) for 2 min in the former and for 7 min in the latter case. In the former case, 25 μg of antimycin A were also present in the preincubation mixture. Suitable aliquots were taken for assaying the enzyme activity. In the case of hypobaria and hypoxia about 40 mg of mitochondrial protein from the control and the exposed animals were used for this experiment. The values represent the means of samples processed individually from 6 animals in each group.

	Activation	*Activity (units/g protein)*			
		Before preincubation with succinate		*After preincubation with 50 mM succinate*	
		Initial	*Twice washed*	*Initial*	*Twice washed*
I.	None	77	60	139	45
	Ubiquinol-9 (0.5 mM)	202	115	181	123
II.	None	54	40	106	54
	Oxaloacetate (0.077 mM)	113	83	102	44
III.	None	57	45	118	47
	Hypobaric	99	87	163	86
	Hypoxic	100	82	134	55

samples obtained under hypobaric and hypoxic conditions showed that the enhanced enzyme activities could not be reversed to the basal levels by washing with the sucrose homogenizing medium (Table V). These mitochondrial samples still retained the enzyme activities at significantly elevated levels as compared to the unexposed controls showing thereby, the effectors responsible for the activation of succinate dehydrogenase under hypobaric and hypoxic conditions may be firmly bound to the enzyme protein. However, a second preincubation of these twice-washed mitochondrial samples with a large excess of succinate followed by two more washings with the sucrose homogenizing medium reversed the enzyme activity to the basal levels in the case of hypoxia. In contrast, the hypobaric activation was not reversed by this washing procedure and retained the enzyme activity at 80% above the control basal activity (Table V). The differential response to the washing procedure observed in the case of hypobaria and hypoxia clearly suggested that despite the activation observed in both the stress conditions, the effectors responsible for bringing about this activation appear to be different in hypobaria and hypoxia. The former activation resembles the ubiquinol-type of activation and the latter, the oxaloacetate–malonate-type.

Effect of exposure to hypobaria and hypoxia on succinate:neotetrazolium reductase activity

Succinate:NT reductase activity which also measures the same flavoprotein as the succinate dehydrogenase, has been shown to be ubiquinone-dependent[12], and to act as a bypass, shunting the electrons off the main pathway of electron transport. This has been shown to increase in the livers of rats exposed to cold stress[13], which is

presumably due to parallel increase in the hepatic ubiquinone content in these cold-exposed animals[14,15]. In the light of these observations, it is proposed that ubiquinone might play a significant role in the extra heat production by driving the electrons towards calorigenic shunt pathways rather than through the slower coupled sequence. These observations lend support to the concept that increase in the succinate:NT reductase activity could represent a measure of the extra heat production.

Exposure of animals to hypobaria or hypoxia for various periods up to 24 h did not result in any appreciable change in the activity of succinate:NT reductase (Table VI). This is interesting in view of the marked stimulation observed in the activity of succinate dehydrogenase. However, on exposure of animals to hypoxic stress for a longer period of 36 h, a significant increase (45%) was observed in the activity of this enzyme and this was not observed in exposure to hypobaria. The differential response in this enzyme activity in animals exposed to hypobaria and hypoxia is interesting in view of the lowered O_2 tension prevailing in both the stress conditions. In view of the hypothermia observed in hypoxic exposures[16], the increased activity in the succinate:NT reductase could represent a compensatory mechanism to overcome the marked heat deficit in the animals exposed to hypoxic stress. This is in agreement with the report that there is a marked drop of 2.5 °C in the rectal temperature of rats exposed to hypoxia for 90 min, whereas in the animals exposed to hypobaric stress for 6 h, the decline in the body temperature is less than 1 °C (ref. 17).

TABLE VI

EFFECT OF EXPOSURE TO HYPOBARIC AND HYPOXIC STRESS ON THE HEPATIC MITOCHONDRIAL SUCCINATE:NEOTETRAZOLIUM REDUCTASE ACTIVITY

The enzyme activity was assayed as mentioned in the text. The values represent the mean ± S.D. of samples processed individually from 8 animals in each group.

	Period of exposure (h)	*Units/g protein*	
		Control	*Exposed*
I.	Exposed to hypobaria		
	2	13.5 ± 2.7	14.7 ± 3.1
	4	11.4 ± 1.6	12.4 ± 3.2
	6	15.2 ± 1.9	14.9 ± 2.7
	9	15.2 ± 1.9	12.2 ± 1.3
	12	8.8 ± 1.4	10.4 ± 3.2
	24	7.4 ± 0.9	7.4 ± 1.0
	36	10.9 ± 1.6	10.7 ± 1.5
II.	Exposed to hypoxia		
	1	10.1 ± 1.5	10.8 ± 1.2
	2	13.5 ± 2.7	12.2 ± 1.8
	4	11.4 ± 1.6	12.2 ± 1.9
	12	8.8 ± 1.3	10.4 ± 3.2
	24	8.8 ± 2.5	7.9 ± 1.8
	36	11.7 ± 1.1	17 ± 4.9*

* Control *vs* exposed, $P < 0.02$.

Reversibility of the enhanced enzyme activity on withdrawal of the stress

To determine whether the increased enzyme activity observed in animals exposed to hypobaric and hypoxic stress could be reverted to the basal levels on with-

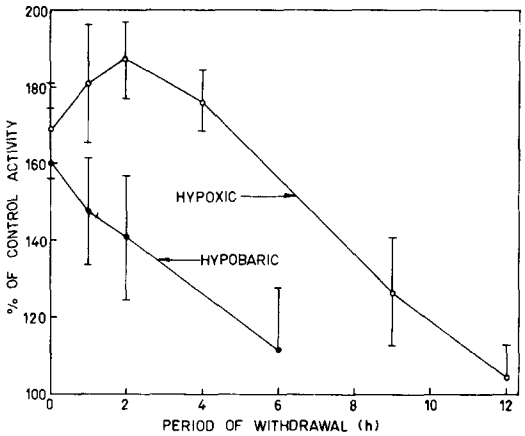

Fig. 3. Effect of withdrawal to atmospheric pressure on the stress-increased hepatic mitochondrial succinate dehydrogenase activity. Rats were exposed to hypobaria or hypoxia for 4 h and then restored to atmospheric pressure for the periods indicated, and the enzyme activities were assayed without preincubation with succinate. The values at each point represent the means ± S.D. of samples processed individually from 6 animals. The values at zero time represent the enzyme activity of animals exposed to the stress for 4 h and is represented as the % of unexposed control activity. At every point the enzyme activities have been represented as the % of the activity in the unexposed control animals.

drawal of the stress, animals were first exposed to hypobaria and hypoxia for 4 h, a period sufficient to bring about the maximal stimulation and then returned to atmospheric pressure and ambient O_2 tension. The basal enzyme activities were determined at various periods of withdrawal of the stress. The data in Fig. 3 showed

TABLE VII

EFFECT OF WITHDRAWAL OF HYPOBARIC AND HYPOXIC STRESS ON THE SUCCINATE DEHYDROGENASE ACTIVITY

The animals were exposed to hypobaria or hypoxia for 4 h prior to withdrawal from the stress. The enzyme activities were assayed with and without succinate as mentioned in the text. The values represent the mean ± S.D. of samples processed individually from 6 animals in each group.

	Period of withdrawal (h)	*Units/g protein*			
		Unexposed control		*Exposed and withdrawn*	
		Basal activity	*Activated with succinate*	*Basal activity*	*Activated with succinate*
I.	Exposed to hypobaria (4 h) prior to withdrawal				
	Initial	40 ± 3	127 ± 9	64 ± 12	123 ± 11
	1	49 ± 6	88 ± 4	72 ± 10	87 ± 9
	2	49 ± 6	88 ± 4	69 ± 11	98 ± 13
	6	56 ± 4	108 ± 17	62 ± 10	86 ± 8
II.	Exposed to hypoxia (4 h) prior to withdrawal				
	Initial	40 ± 3	127 ± 9	69 ± 9	138 ± 14
	1	52 ± 3	95 ± 12	94 ± 16	133 ± 12
	2	52 ± 3	95 ± 12	98 ± 11	135 ± 6
	4	52 ± 3	95 ± 10	92 ± 7	129 ± 22
	9	53 ± 7	88 ± 10	67 ± 10	111 ± 10
	12	53 ± 5	97 ± 11	58 ± 5	99 ± 9

that the enzyme activity which was about 60–70% above the control levels in the exposed groups reverted to the basal levels on withdrawal of the stress in both cases. Withdrawal of the hypobaric stress resulted in a rapid loss of about 30% of the enhanced enzyme activity in a short period of 2 h and at 6 h the activity reached the basal level. However, on withdrawal of the hypoxic stress, there was no significant decline in the enzyme activity up to 2 h and in fact a slight stimulation of the activity was observed. A rapid decline in the enzyme activity was observed subsequently and a complete reversal to the basal level was observed at 12 h. The above-mentioned pattern of reversal of the enzyme activity was true only when basal activities of the enzyme were assayed without preincubation. However, preincubation of the mitochondrial samples with succinate gave the same maximal activity for the control and the hypobaric and hypoxic activated samples (Table VII), confirming that the amount of the enzyme protein remained constant.

DISCUSSION

The physiological and biochemical mechanisms that govern the adaptation of animals to environmental stress like hypoxia and hypobaria are not clear. Exposure to both the conditions of stress could result in a lowered O_2 tension in the tissues, thus seriously affecting many metabolic processes dependent on molecular O_2, including energy production. The physiological responses to these stress conditions favours the development of compensatory mechanisms to overcome the effects of the stress.

Increase in cellular oxidations as well as in the activities of several oxidative enzymes has been reported in several tissues of animals after acute and chronic exposure to hypoxic stress[18–22]. Of these, the increase in the rate-limiting steps would be of particular importance. As observed in the present study, the modulation of the rate-limiting step catalyzed by succinate dehydrogenase could result in an increase in the overall rate of oxidation of succinate and represent a compensatory mechanism directed towards overcoming the initial lack of O_2 and provide for the minimal energy requirements. The reversal of the enzyme activity to control levels on withdrawal of the stress also supports this concept. Thus the specific increase in the activity of succinate dehydrogenase in the livers of rats exposed to hypobaria and hypoxia could be of physiological significance to the animal.

Although the physiological responses obtained in hypobaria have often been ascribed to the prevailing hypoxia, reduced pressure *per se* has been reported to elicit specific responses in the activities of certain enzymes[2] and other metabolic reactions[23] which are not observed in hypoxia. Our studies suggest that although the initial responses elicited in the activity of succinate dehydrogenase in hypobaria and hypoxia were apparently similar, longer exposures resulted in the stabilization of the enzyme activity at a new steady state level in the latter case, but reverted to the basal level in the former. These observations also indicate that the factors responsible for the observed changes are different in the two conditions.

The nature of the activation observed under hypobaria and hypoxia deserves some comment. Physical exposure of intact animals for short periods to low pressure or O_2 tension seems to result in structural alterations of the enzyme protein in the hepatic mitochondria. Succinate dehydrogenase is known to exist in a basal and an

activated state. This activation phenomenon is the result of a conformational change at the molecular level, obtained on preincubation of mitochondria with succinate[10] and a variety of effectors[3]. The activation is very rapid at 37 °C and requires only very small concentrations of the effectors. The conditions prevailing in the cell, particularly the temperature, are conducive for the activation to occur provided sufficient concentrations of some of the naturally occurring effectors are available. Attractive theories have been proposed for the control aspects of this activation phenomenon based on studies *in vitro*. The physiological demonstration of such activated enzyme systems under hypobaric and hypoxic conditions in the present investigation establishes the validity of these hypotheses.

Kinetic studies on the effect of succinate concentration on the activated enzyme preparations obtained from animals exposed to hypoxia, indicated that both the K_m for succinate and V increased suggesting that this activation did not fall into the classical K or V systems suggested by Monod *et al.*[24]. Studies on the nature of the effector responsible for the activation in hypobaria and hypoxia, by studying the reversibility to washing, as well as the lack of stimulation on the succinate:NT reductase activity, suggested that the former resembles the ubiquinol-type and the latter may be of the dicarboxylate-type (Table VIII).

TABLE VIII

A COMPARISON OF THE ACTIVATION OF SUCCINATE DEHYDROGENASE BY EFFECTORS *IN VITRO* AND IN HYPOBARIA AND HYPOXIA

For activities: increased, +; no change, −. For washing: reversed, +; not reversed, −.

Type	*Effector*	*Succinate:PMS reductase*	*Succinate:NT reductase*	*Reversal of activation on washing*	
				Without succinate	*With succinate*
Dicarboxylate	Malonate	+	−	−	+
	Oxaloacetate	+	−	−	+
Quinols	Ubiquinol-9	+	−	−	−
Pyrophosphates	Pyrophosphate	+	+	−	+
Low pressure and low O_2 stress	Hypobaria (4 h)	+	−	−	−
	Hypobaria (36 h)	+	−	−	−
Low O_2 stress	Hypoxia (4 h)	+	−	−	+
	Hypoxia (36 h)	+	+	−	+

The increase in the enzyme activity observed in hypobaria and hypoxia appears to be specific for succinate dehydrogenase, since succinate:NT reductase activity which also shares the same flavoprotein as the dehydrogenase is unaffected by exposure to hypobaria or hypoxia for short periods. This observation invokes the possibility of a differential modification of the dye acceptor sites in conditions of stress. Quite a few evidences are now available for the role of this enzyme system in the production of extra heat under conditions where there is a physiological demand such as cold exposure[13]. Our results on longer exposures to hypoxic stress showed

that succinate:NT reductase activity increased considerably in hypoxia but not in hypobaria. This is interesting in view of the marked heat deficit on exposure to hypoxia[16,17]. Such a simultaneous increase in the activities of succinate dehydrogenase and succinate:NT reductase can be obtained by other effectors, *e.g.* pyrophosphates[3] (Table VIII). It seems likely that chronic exposure to hypoxia evokes a combination of effects necessary for the adaptation process.

ACKNOWLEDGEMENT

This work forms part of the project on Environmental Stress and Biochemical Adaptation sponsored by Office of Naval Research (U.S.) under contract No. N0014-71-C-0348. We thank Miss K. Sathyavathy for technical assistance.

REFERENCES

1 Aithal, H. N. and Ramasarma, T. (1969) *Biochem. J.* 115, 77–83
2 Susheela, L., Inamdar, A. R. and Ramasarma, T. (1972) *FEBS Lett.* 25, 295–297
3 Susheela, L. and Ramasarma, T. (1973) *Biochim. Biophys. Acta* 292, 50–63
4 Susheela, L. and Ramasarma, T. (1971) *Biochim. Biophys. Acta* 242, 532–540
5 Arrigoni, O. and Singer, T. P. (1962) *Nature* 193, 1256–1258
6 Slater, T. F. (1963) *Biochim. Biophys. Acta* 77, 365–382
7 Potter, V. R. (1957) in *Manometric Techniques*, (Umbreit, W. W., Burris, R. H. and Stauffer, J. F., eds.), p. 170, Burgess Publishing Co, Minneapolis
8 Tisdale, H. D. (1967) in *Methods in Enzymology*, (Estabrook, R. W. and Pullman, M. E., eds), Vol. 10, pp. 213–215, Academic Press, New York and London
9 Kimura, T., Hauber, J. and Singer, T. P. (1967) *J. Biol. Chem.* 242, 4987–4993
10 Kearney, E. B. (1957) *J. Biol. Chem.* 229, 363–375
11 Gonzalez-Cadavid, N. F. and Campbell, P. N. (1967) *Biochem. J.* 105, 443–450
12 Lester R. L. and Smith, A. L. (1961) *Biochim. Biophys. Acta* 47, 475–496
13 Aithal, H. N. and Ramasarma, T. (1971) *Biochem. J.* 123, 677–682
14 Beyer, R. E., Noble, W. M. and Herschfeld, T. J. (1962) *Can. J. Biochem. Physiol.* 40, 511–518
15 Aithal, H. N., Joshi, V. C. and Ramasarma, T. (1968) *Biochim. Biophys. Acta* 162, 66–72
16 Bhatia, B., George, S. and Rao, T. L. (1969) *J. Appl. Physiol.* 27, 583–586
17 Defeo, J. J., Baumel, I. and Lal, H. (1970) *Fed. Proc.* 29, 1985–1990
18 Clark, Jr, R. T., Chinn, H. I., Ellis, Jr, J. P., Pawel, N. E. R. and Criscuolo, D. (1954) *Am. J. Physiol.* 177, 207–210
19 Tappan, D. V., Reynafarge, B., Potter, V. R. and Hurtado, A. (1957) *Am. J. Physiol.* 190, 93–98
20 Chinn, K. S. K. and Hannon, J. P. (1969) *Fed. Proc.* 28, 944–947
21 Woodhall, B., Kramer, R. S., Currie, W. D. and Sanders, A. P. (1971) *J. Neurosurg.* 34, 3–14
22 Shertzer, H. G. and Cascarano, J. (1972) *Am. J. Physiol.* 223, 632–636
23 Bernardini, A. T. (1969) *Fed. Proc.* 28, 1163–1169
24 Monod, J., Wyman, L. and Changeux, J. P. (1965) *J. Mol. Biol.* 12, 88–118