

## CHANGES IN THE ACTIVITIES OF TWO MEMBRANE-BOUND ENZYMES DURING SOLAR ECLIPSE ON FEBRUARY 16, 1980

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ON Saturday February 16, 1980 total solar eclipse occurred for a period of 2–3 min in a belt of 135 km during the eclipse from 14·17 to 17·00 hrs across peninsular India. The city of Bangalore, being just outside this belt, had witnessed 92% eclipse for about 2½ min at the peak period of 15·44 hr at which time a temperature drop of 2°C and a considerable dimness of the light were experienced. In view of the interest in our laboratory on biochemical adaptation under conditions of environmental stress, we designed an experiment to study the possible changes in enzyme activities during the solar eclipse on February 16, 1980.

Groups of male Wistar albino rats (weight range 120 ± 5 g, four per group) were provided with food and water *ad libitum* and kept in separate cages in an enclosed corridor where the animals were fully exposed to sunlight from 10·00 hrs onwards. The same experiment was carried out on February 15 and February 16, the former serving as the control for the effect of solar eclipse. There was no difference in the food intake in the animals on the two days of the experiments. One group of animals was killed by cervical dislocation at each of the time intervals—12·30, 14·30, 16·00 and 18·30 hrs—on each day and blood and liver were taken for analysis. The following four oxidative enzymes were tested:

### 1. *NADH oxidase of erythrocyte membranes*

The enzyme, NADH oxidase (insensitive to rotenone, antimycin A and cyanide and therefore distinct from the mitochondrial type) is found in a number of plasma membranes of a variety of cells<sup>1</sup>. This enzyme in erythrocyte membrane has very low activity with oxygen but shows good rate with ferricyanide as the acceptor<sup>2</sup>. The low activity with oxygen of this enzyme in rat erythrocyte membranes was found to be stimulated about 50-fold on addition of 0·16 mM of decavanadate. This enzyme is considered to have a role in transport processes<sup>3</sup>.

Erythrocyte membranes were prepared from the sedimented red blood cells according to the method of Dodge *et al.*<sup>4</sup>. The enzyme activity was measured by the decrease in the absorbance of NADH at 340 nm in a reaction mixture containing 0·1 mM of NADH and 0·1 mg protein of erythrocyte membranes in 1·0 ml of phosphate buffer (pH 7·0, 0·05 M).

### 2. *Vanadate-stimulated NADH oxidase of microsomes*

Like the plasma membrane enzyme, hepatic microsomes also possess a distinctive NADH oxidase whose activity is high with ferricyanide as acceptor but low with oxygen that can be stimulated 250-fold by 0·1 mM decavanadate. This activity which will result in converting energy of oxidation into heat may have a role in thermogenesis.

Microsomes were prepared from livers by the method of Shapiro and Rodwell<sup>5</sup>. The oxidation of NADH was measured by the uptake of oxygen with a Clark electrode in a Gilson Oxygraph. The reaction mixture contained phosphate buffer (pH 7·0, 0·05 M), 0·1 mM of NADH and 0·2 mg microsomal protein in a total volume of 1·5 ml.

### 3. *Tryptophan pyrrolase of cytosol*

Tryptophan pyrrolase degrades tryptophan by oxidizing it to N-formylkynurenine which is then converted to kynurenine. This enzyme is inducible by cortisol and tryptophan, has a short half-life of 90 min and exhibits a circadian rhythmic peak activity at night<sup>6</sup>. Hepatic tryptophan pyrrolase is inversely related to brain serotonin<sup>7</sup>.

The enzyme was measured in 3600 × g supernatants of 1·15% KCl homogenates of livers by the method of Knox and Aurebach<sup>8</sup> with the modification of preincubation according to Sitaramam and Ramasarma<sup>9</sup>. The incubation mixture consisted of 2 μ moles of L-tryptophan, 50 μg of haematin and phosphate buffer (pH 7·0, 0·1 M) in a total volume of 2 ml. After the initial preincubation for 30 min at 37°C, 10 μ moles of L-tryptophan were added and the reaction was carried out for 20 min. The kynurenine formed was estimated by absorbance at 365 nm.

### 4. *3-Hydroxy-3-methylglutaryl (HMG) CoA reductase of microsomes*

HMGCoA reductase is responsible for the formation of mevalonate, a key intermediate in isoprene biosynthesis<sup>10</sup>. This is the rate limiting step in cholesterol biosynthetic pathway in the liver. It has a half-life of about 3 hr and exhibits circadian rhythmic peak activity of midnight<sup>11</sup>. It decreases rapidly on deprivation of food and increases on treatment with noradrenaline<sup>12</sup> and adenosine<sup>13</sup> compounds. It is inhibited *in vitro* by vanadium,

The enzyme activity was measured in microsomes prepared from livers as described above. The reaction mixture consisted of  $^{14}\text{C}$ -HMGCoA (0.5 mM, 365 cpm/nanomole), dithiothreitol (10 mM), NADP (3.65 mM), G-6-P (4.5 mM), G-6-P dehydrogenase (0.5 units) and phosphate buffer (pH 7.4, 50 mM) in a total volume of 0.1 ml. After incubation for 20 min at 37°C, 30  $\mu\text{l}$  of 10N HCl was added to stop the reaction and the mixture was then incubated at 37°C for 30 min to lactonize mevalonate formed. The mixture was separated on silica gel t.l.c. and the radioactivity in mevalonolactone was measured<sup>12</sup>.

In this set of experiments the animals tested on February 15 were considered to be the appropriate controls for those on February 16 as they were treated identically. The results presented in Fig. 1 are the mean  $\pm$  S.D. values of independent analysis of the animals in each group. The results, analyzed statistically, by Student's 't' test, show that two enzymes have changed on February 16 as compared to February 15. The actual values in the groups at 16:00 hrs are given in Table I.

The erythrocyte membrane NADH oxidase showed a remarkable stimulation at 16:00 hrs, just after the peak period of eclipse. The activity is always very low in the controls tested many times before and after February 16 and all the animals in the group at 16:00 hrs on February 16 responded to this stimulation (Table I). This enzyme measured by reduction of ferricyanide shows much higher rate but this ferricyanide-dependent activity remained unchanged on February 15 and February 16. Thus, only the sensitivity to oxygen as the electron acceptor increased on February 16. Such an effect is obtained when micromolar concentrations of decavanadate were added<sup>14</sup>.

Similar experiments were carried out on February 25 at 16:00 and 20:00 hrs and no difference in any of these activities was found at these two time intervals. Since the 20:00 hrs group represents the control for both decreased temperature and light, the effect on February 16 cannot be ascribed to these physical parameters.

NADH oxidase in hepatic microsomes, however, was unaffected. The cytosolic tryptophan pyrrolase also showed no change.

The hepatic microsomal HMGCoA reductase showed a decrease of about 50% at 16:00 hr on February 16 compared to that on the previous day.

Of the two NADH oxidases only the erythrocyte enzyme increased. Of the two circadian enzymes only the HMGCoA reductase decreased. It appears that the above effects may have resulted due to disturbance in some radiations on the eclipse day. These observations, however, prompt further studies on biochemical changes in animals exposed to specific radiations.

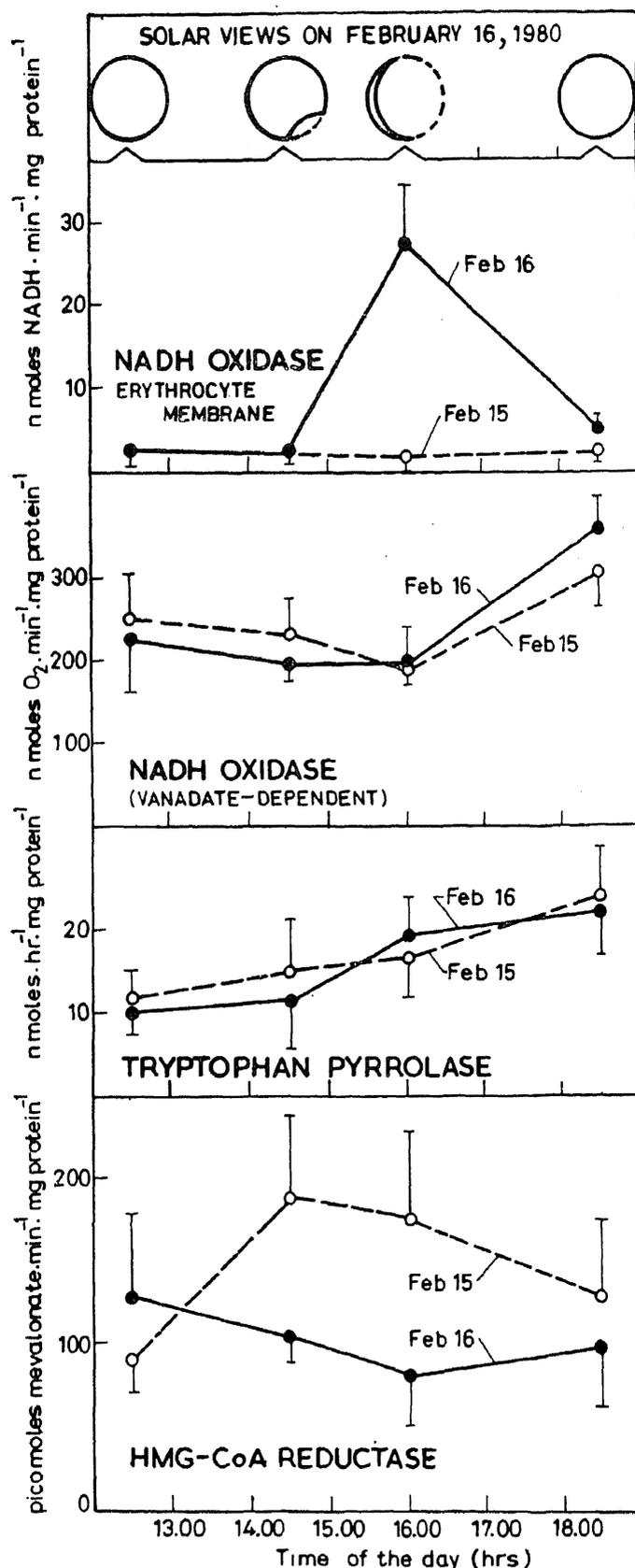


FIG. 1

ACKNOWLEDGEMENT

Financial assistance from the Indian Council of Medical Research for the project on Rhythms in Isoprene Biogenesis, from the Indian National Science Academy for the project on Cellular Organelles and from the University Grants Commission for the projects on Environmental Stress and Biochemical

TABLE I

Individual values of enzyme activity in groups of animals on Feb. 15 and Feb. 16 at the 16.00 hr time interval

Enzyme (units)	Enzyme activity							
	Feb. 15				Feb. 16			
NADH oxidase of erythrocyte membrane* (nmoles NADH. min <sup>-1</sup> . mg protein <sup>-1</sup> )	3.2,	1.6,	1.1,	31.7,	31.7,	19.0,		
NADH oxidase of hepatic microsomes (nmoles O <sub>2</sub> . min <sup>-1</sup> . mg protein <sup>-1</sup> )	242,	124,	174,	212	213,	168,	206,	190
Tryptophan pyrrolase of hepatic cytosol (nmoles. hr <sup>-1</sup> . mg protein <sup>-1</sup> )	14.1,	26.4,	12.0,	18.3	20.1,	18.2,	27.7,	12.2,
HMGCoA reductase of hepatic microsomes (pmoles mavalonate. min <sup>-1</sup> . mg protein <sup>-1</sup> )	130,	214,	115,	210	115,	110,	71,	69

\* Only three samples were tested.

Adaptation II and Influence of Drugs on Mitochondrial Metabolism, and the Junior Research Fellowship to S. V. from the Department of Atomic Energy are acknowledged.

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## SORGHUM BASED CROPPING SYSTEMS TO MEET SHORTAGES OF PULSES AND EDIBLE OILSEEDS

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### ABSTRACT

The shift from tall and late varieties of sorghum to early maturing hybrids has not only conferred greater levels of productivity and stability but has opened up opportunities for practice of new cropping systems in rainfed areas. Based on extensive experimentation, the opportunities for enhancing pulse and edible oilseed production on the existing areas of sorghum through practice of suitable inter- and sequence cropping systems have been analysed.

**T**RADITIONAL tropical dryland agriculture continues to be largely of the subsistence type. Tropical cultivars are generally tall and late (>150 days), the duration of the crop growing season being longer

compared to the duration of rainy season. Subnormal rains or restricted duration of monsoon result in the reduction of yields or total crop failures (Rao *et al.*<sup>3</sup>). Traditional mixed cropping, practised over ages, has