azafluoranthene and palladium on calcium carbonate, anthracene gives no hydrogen; fluorene gives 0.64 mole of hydrogen per mole of hydrocarbon.

Bruceton vitrain has thus been found to evolve about one-third of its hydrogen when dehydrogenated in the presence of phenanthridine, and about one-half when dehydrogenated in 2-azafluoranthene. Some of the hydrogen evolved with 2-azafluoranthene is probably due to side reactions; nevertheless, we believe that the hydroaromatic hydrogen in Bruceton vitrain is at least 30 percent, and may be considerably higher.

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Physiology of Acclimation to Low Temperature in Poikilotherms

Abstract. Potassium, sodium, and calcium increase and chloride, magnesium, and free amino acids decrease in cold acclimated fresh-water mussels and earthworms. Increased protein synthesis occurs. Neurosecretory cells of cold earthworms exhibit increased activity. Addition of cold worm body fluid stimulates increased O_2 consumption by normal tissues, indicating that a hormonal agent triggers the sequence of changes.

Since the pioneering studies of Fox (*1*), considerable evidence has accumulated which shows that many poikilotherms are able to compensate for temperature in their metabolism and activity (*2*). The degree of compensation is different in different groups of animals (*2, 3*) and the mechanisms underlying this compensation are not fully understood. It has been shown that the quantity of bound water changes (*4*). Several studies indicate that enzyme activity changes (*4, 5*). Changes in metabolic pathways have even been suggested (*5*). Pampapathi Rao and Ramachandra (*6*) reported recently that acclimation to high temperature results in changes in the level of free amino acids in the blood and body fluids of some invertebrates. We have, therefore, undertaken to find out the causes and consequences of the change in amino acid level in the hope of understanding the mechanisms underlying temperature acclimation. The present paper is a preliminary report of the results obtained from our study of cold acclimation in two tropical poikilotherms.

Table 1. Changes in the concentration of free amino acids and certain inorganic ions in the blood of the fresh-water mussel and the body fluids of the earthworm, after acclimation to low temperature. In all cases the data are based on an analysis of not less than 18 individuals. All figures are in millimoles per liter, except for the concentration of free amino acid, which is expressed as mg/100 ml.

State of acclimation	Free amino acids	Cl	Mg	K	Na	Ca
		Mussel, Lamellidens marginalis				
Normal 29°C	4.03 ± 0.5	14.7 ± 1.6	—	0.62 ± 0.04	18.8 ± 1.2	3.37 ± 0.37
Cold 19°C	2.3 ± 0.3	10.2 ± 1.0	—	0.73 ± 0.05	22.5 ± 1.4	5.63 ± 0.62
		Earthworm, Lampito mauritii				
Normal 29°C	104 ± 28	36.7 ± 4	8.9 ± 1	11.9 ± 3	38.5 ± 8	9.16 ± 1.2
Cold 19°C	56.2 ± 20	31.7 ± 4	6.5 ± 1.3	17.5 ± 3.5	49.8 ± 9	12.6 ± 2

The freshwater mussel, *Lamellidens marginalis,* and the earthworm *Lampito mauritii* were used in this investigation. The normal (room) temperature during these studies was 29° ± 1°C. In addition to animals kept at room temperature, several batches of individuals of both species were acclimated to 19° ± 1°C for 20 to 30 days in the laboratory. Blood of mussels (drawn directly from the heart) and body fluids of earthworms were analyzed for chloride, sodium, potassium, calcium, magnesium, and free amino acids in the normal as well as cold acclimated animals. There was an increase in sodium and potassium during cold acclimation in both species, while chloride, magnesium, and free amino acids decreased during cold acclimation. Calcium increased markedly in cold mussels but in cold earthworms the increase was not as great. Because potassium activates muscle metabolism (*7*), an increase in potassium in body fluids of cold acclimated forms will tend to increase muscle metabolism and this effect will be augmented when the calcium concentration also increases. These effects are further enhanced by the simultaneous decrease in magnesium (see Table 1).

An interesting result is the decrease in free amino acids in cold acclimated individuals. It was thought that this decrease might result from increased incorporation of amino acids into proteins of the cells, due to increased protein synthesis during cold acclimation. Individual blood cells in cold acclimated mussels were measured under the interference microscope (*8*) for total protein content (dry matter) and compared to those of normal individuals. There was a 21 percent increase in the protein content of the cells of cold acclimated individuals.

Nucleic acid content of different tissues of several normal and cold acclimated mussels was estimated by the spectrophotometric method of Spirin (*9*). The concentration of nucleic acid phosphorus in the hepatopancreas of cold acclimated mussels doubled compared to normal, but in the foot and ctenidia the increase was not so noticeable. These results show that metabolically active tissue (hepatopancreas) exhibits a great increase in nucleic acid content, implying increased protein synthesis, which is also indicated by the increased protein content of the cells.

The sequence of systemic changes, increased protein synthesis, and increased nucleic acid recall the sequence of events preceding molting of insects. Because the process in insects is triggered by hormonal agencies arising in neurosecretory cells, an attempt was made to localize such agencies in earthworms. The supra- and subesophageal ganglia of normal and cold earthworms were sectioned at 6 μ in paraffin and stained in Gomori's chrome alum haematoxylin-phloxine to show neurosecretory cells. As judged by the intensity of staining and dense packing of particles, there is a distinct increase in the activity of neurosecretory cells in cold acclimated individuals. This increase suggests the possibility that a hormonal product of neurosecretion plays an important role either by triggering the events that cause metabolic compensation through systemic changes and increased protein synthesis, or by directly influencing tissue metabolism,

or both. We obtained evidence for a factor borne by blood or body fluid which increases metabolism of cold acclimated worms. A small quantity of body fluids from cold acclimated worms was added to a Warburg flask containing tissues from the muscle of the body wall of normal worms. Oxygen consumption of the tissues increased by 25 percent compared to normal tissues with an equal quantity of body fluid from normal worms, as measured simultaneously in the same Warburg bath.

The sequence of events in cold acclimation, then, appear to be triggered by the release of a neurosecretory product which results in increased protein synthesis (perhaps resulting in increased concentration of metabolic enzymes in the cells) and ionic changes which help to increase muscle metabolism (activity).

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Formation of Carbon Monoxide during Seed Germination and Seedling Growth

Abstract. Carbon monoxide was formed during the growth of cucumber seedlings in the dark in atmospheres containing 5 percent oxygen or less, but not by aerobic seedlings. The highest level recorded was 6000 ppm. Carbon monoxide was also formed by *Euphorbia.* Germinating seeds of rye, cucumber, and other species also produced carbon monoxide at levels of 10 to 25 ppm.

In the course of a recently initiated study of plant behavior at subatmospheric oxygen levels (*1–3*) it was observed that the available O_2 was inadequate to account for measured loss of carbon as CO_2. A more reduced metabolite was sought, and, when aldehyde tests failed to yield a satisfactory result, analyses were carried out for carbon monoxide. We now report our observations on CO production, especially during germination and seedling growth (*4*).

Routine determinations of CO were made on an M.S.A. carbon monoxide tester with indicating tubes (*5*) over its useful range of 2.5 to 1000 ppm. The indicator tube measurements were supported by standard gas chromatographic procedures (Beckman model GC-2), which also permit measurements in a far higher range.

Carbon monoxide was first detected in the atmosphere of cucumber seedlings which had been grown from seed at 25°C in "Perl-lome" (*6*) in a 5 percent oxygen plus 95 percent argon atmosphere. The atmosphere in sealed growth jars as previously described (*2*) was adjusted to 5.0 percent O_2, 0.002 percent CO_2 after 4 days' incubation, and the jars were placed in darkness for 7 days.

At the end of this period, the O_2 content was unchanged, and CO_2 had risen to about 3.5 percent. The indicator tube method showed that the atmosphere contained far more than 1000 ppm CO, and gas chromatography showed approximately 6000 ppm. (Neither "Perl-lome" nor "Perl-lome" and soil mixtures generated detectable CO.) The jars (gas volume, 7000 cm^3) contained 38 completely achlorophyllous seedlings totaling 12 g in fresh weight. Accordingly, some 4.2 mg of CO per gram of fresh weight had been generated. It should also be noted that the 38 seedlings doubled in height during this period, elongating some 30 mm on the average. No aldehydes were detected with Schiff's reagent either in the jar atmosphere or the substratum.

In a subsequent experiment, cucumber seeds were germinated in 5 percent oxygen plus 95 percent argon and, after 4 days, 12 seedlings, 5 to 6 mm high, were transferred to an atmosphere containing < 0.5 percent O_2 and about 0.24 percent CO_2 in argon. After 10 days in darkness, when the seedlings had increased about 10 mm in height, their atmosphere showed no change in O_2, increased CO_2 (1.5 percent), and 0.04 percent (400 ppm) CO. During an additional 8 days in darkness, the seedlings increased approximately 20 mm more in height. At that time, oxygen in the atmosphere continued unchanged, but CO_2 had increased to more than 5 percent, and CO had fallen to 10 ppm.

Table 1. Carbon monoxide production by seeds after 5 days at reduced oxygen levels. Schiff test for aldehydes: N, negative; P, positive; PP, intense positive; W, weak.

Seed and wt. of seed used (g)		CO level		Schiff test
		ppm	μg/g seed	
Rye	(39)	25	1.95	N
Maize	(44)	0		W
Pea	(44)	10	0.36	P
Bean	(42)	0		W
Tomato	(14)	0		N
Cucumber	(34)	15	1.04	P
Turnip	(35)	10	0.69	N
Lettuce	(24)	10	0.48	PP

In contrast to the foregoing, cucumber seedlings which had been grown in air produce no detectable CO, whether maintained in air or placed for as long as 7 days in low oxygen.

Euphorbia clandestina can produce CO. Four plants totaling 50 g in fresh weight were maintained for about 3 months in sealed containers in a greenhouse with a diurnal cycle from +20°C (max.) to −10°C (min.) The initial atmosphere consisted of 0.09 percent O_2, 0.24 percent CO_2, 1.4 percent argon, and N_2 to give $P_{total} = 0.1$ atm. At the end of the test period, O_2 measured 12 percent, $CO_2 > 5$ percent. The 16-liter atmosphere in this experiment contained 10 ppm CO, or about 200 μg in total. No gases other than those mentioned were found by gas chromatography, although H_2, CH_4, and aldehydes would have been detected if present. *Euphorbia* plants maintained at initially higher oxygen levels, on the other hand, gave no detectable CO.

The production of CO by seeds has also been demonstrated. Approximately 50 cm^3 of loosely packed seeds and an equal volume of oxygen-free water were introduced into 500-cm^3 polyethylene vessels, and the vessels were evacuated, filled with 5 percent oxygen plus 95 percent argon and held at 25°C. After 2 days, winter rye, Alaska peas, Marketer cucumber, Purple Top White Globe turnip, and Black Seeded Simpson lettuce had formed traces of CO, whereas Golden Bantam corn, Red Kidney bean, and Marglobe tomato had not. The same results were obtained whether or not the seeds were sterilized in 0.5 percent NaOCl. After 5 days, appreciable quantities of CO were present in the atmospheres of several species (Table 1). Schiff tests were made, but the results were too variable to suggest a relationship between aldehydes and CO.

Recently, Wilks (*7*) has reviewed the