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

rather sparse literature dealing with CO in plants. From his own experiments he has concluded that light, chlorophyll, and O_2 together are responsible for the concurrent production of CO and aldehydes via a photodegradative process. He further associates injured tissues particularly with CO formation.

Our experiments show that neither light nor chlorophyll is necessary for CO formation, and that, although some oxygen may be required, high levels do not favor its production. Carbon monoxide is formed both by seeds and intact, growing plants. Such results suggest the operation of novel fermentations in higher plants and constitute an extension rather than a contradiction of Wilks's observations.

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Equilibration of Isoagglutinins of Human Group O Serum

Abstract. Cross-reactive antibodies are shown to be responsibile for the difference in final levels of agglutination reached after dissociation of a centrifuged group O serum-erythrocyte mixture as compared with duplicate mixtures allowed to aggregate freely. This difference is seen only with certain group O sera, it is independent of complement, and it may be eliminated by absorption of the serum with A or B erythrocytes. The phenomenon is most likely the result of steric inhibition of smaller, cross-reactive antibodies in the aggregative system.

When equal volumes of an antiserum dilution and a suspension of red blood cells are mixed and allowed to aggregate freely (association), the percentage of agglutinated cells eventually reaches a stable maximum level. A final maximum response is also achieved when a duplicate reaction mixture is first centrifuged and the sedimented cells are dispersed during continuous agitation (dissociation). The system is considered to have attained a true and stable equilibrium when the percentage of agglutinated cells is the same by the two methods (*1*).

Isoagglutinins from unstimulated group A and group B persons characteristically reach a true equilibrium when they are allowed to react with group B and group A cells respectively. The equilibration curves for the isoantibodies of many unstimulated group O sera, however, show a consistent lack of true equilibrium even though stable levels of agglutination are produced by both the associative and dissociative methods. Quantitative hemagglutination assays can be successfully accomplished only when the system has reached a stable equilibrium. It was considered worthwhile to determine which, if either, of the two levels obtained with group O serum represents a true measure of agglutinating antibody. It appears that the dissociative method is preferable when a measure of total antibody is required.

Sera were obtained from male and nulliparous female donors with no history of recent immunization. Each serum was tested for cross-reactive antibody by the mixed-cell agglutination method of Jones and Silver (*2*). Equilibrium studies were performed by mixing 0.5 ml of a dilution of antiserum that would produce about 80 percent agglutination with a suspension of red blood cells in 0.25 percent saline. The resulting mixture contained 1.0 to 1.3 $\times 10^4$ cells per cubic millimeter. Unagglutinated cell controls were prepared by substituting saline for the serum dilution.

The serum-cell mixtures for the associative and dissociative assays were prepared identically. In the dissociative assay, the tubes were centrifuged for 1 minute at 200 grav and were then placed along the circumference of a Dacie agitator wheel (*3*) 11 inches in diameter and rotating at a constant speed of 10 rev/min. The same procedure was followed in the associative assay except that centrifugation was omitted.

Quadruplicate hemocytometer counts of the number of free cells were made periodically from duplicate tubes obtained from each of the two assays. Tubes were sampled once and discarded.

The results with one serum are shown in Fig. 1. This serum was representative of 11 tested and was selected for illustration because the titers with A and B

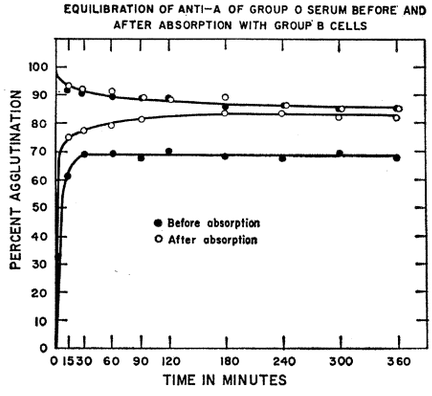

Fig. 1. Equilibration of anti-A of group O serum before and after absorption with group B cells. Note also that dissociation (upper curve) proceeds at the same rate before and after absorption while association is more rapid after absorption (middle curve) than before (lower curve).

cells were equal, avidity times were the same, and cross-reactivity was detectable. Since the results with A and B cells were similar, only those obtained with A cells are given for simplicity. The untreated serum had an anti-A titer of 32 units by the customary serial dilution titration. The slide avidity time at this dilution was 5 seconds. An antiserum concentration of 0.0312 ml/ml was employed in the two assays and gave values of 69 percent agglutination in the associative assay and 84.7 percent in the dissociative assay.

After seven absorptions with one-half volume of packed, washed group B cells, the anti-B activity was completely removed and the anti-A titer was reduced to 8 units. The avidity time was increased to 12 seconds after absorption. Sera absorbed with group O cells gave the same results as unabsorbed sera.

Equilibrium studies with the absorbed serum (0.125 ml/ml) showed that the system could now attain an essentially true equilibrium. As shown in Fig. 1, endpoint values for the assays were within 2 percent of identity. An important feature to be noted is that dissociation follows the same course with absorbed and unabsorbed sera and results in identical curves.

In order to rule out any effects of complement, fresh sera were compared with sera in which complement activity had been removed. Two methods were used for decomplementation: (i) heat treatment at 63°C for 10 minutes and (ii) absorption with a washed immune precipitate prepared from the reaction of crystalline bovine serum albumin (BSA) with rabbit anti-BSA (*4*). In each