of development. However, it is possible that, like leucine, little uptake of methionine occurs at this time, and thus, their method is not sufficiently sensitive to detect the small amount of protein synthesis demonstrated by CO_2 fixation and the incorporation of lysine.

An important question concerns the role that CO_2 fixation plays in the normal development of embryos in their natural physiological environment. All embryos are exposed to CO_2 in early development, but the environmental concentrations vary considerably. Oviparous forms develop in an aquatic environment, where CO_2 exists as bicarbonate and carbonate ions, carbonic acid, and undissociated molecules of CO_2. The total CO_2 content of seawater, for example, is approximately 2.0 mmole per liter (*13*).

Analyses of the secretions of the oviducts in the rabbit and sheep show that much higher concentrations of bicarbonate occur. These concentrations are close to those found in blood (*14*) and are about 10 to 15 times higher than in seawater. Thus, at the time of fertilization and early development, mammalian embryos, unlike aquatic forms, are exposed to an environment that is rich in bicarbonate, thereby providing ample opportunity for CO_2 fixation. The known importance of bicarbonate in mediums used to culture mouse embryos at stages prior to implantation suggests that CO_2 is essential for early development in this species. Moreover, in the sheep, the bicarbonate content of the oviduct fluid is higher during estrus than it is during diestrus, and it is elevated in ovariectomized ewes after the injection of 17β-estradiol (*2*). Perhaps, during the evolution of viviparity in mammals, a hormonally controlled mechanism for enhancing the content of bicarbonate in the oviductal secretions has been selected.

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15. Work done while C.N.G. was on leave from the department of dairy science, University of Illinois, as a special NIH fellow. The work was also supported in part by grants from the Ford Foundation, National Institute of Child Health and Human Development, and the Population Council.

23 October 1969; revised 12 December 1969

Hyperbaric Oxygen: Effects on Metabolism and Ionic Movement in Cerebral Cortex Slices

Abstract. *The incubation of slices of cerebral cortex under hyperbaric oxygen pressures from 1 to 10 atmospheres in the presence of radioactive glucose, pyruvate, succinate, fumarate,* L-*glutamate, and gamma-aminobutyric acid causes a marked diminution of tissue oxidative reactions. There is a simultaneous decrease in phosphocreatine and adenosine triphosphate, and a reduction of the apparent intracellular ionic gradients. The increase of lipid peroxides, measured directly, is attributed to the toxic effects of hyperbaric oxygen.*

Exposure of man and animals to oxygen at high pressure induces generalized convulsions similar to epileptic fits (*1, 2*). In man, convulsions occur after a latent period which is inversely proportional to the partial pressure of oxygen (*3*). Also, hyperbaric oxygen has an inhibiting action on numerous enzymes (*4*) and lowers the oxygen uptake of brain slices and homogenates (*5*). The onset of convulsions appears to occur before significant alteration of metabolic reactions (*1, 4, 6*). We have attempted to determine whether hyperbaric oxygen alters oxygen uptake in guinea pig cerebral cortex slices incubated in Krebs-Ringer glucose saline solution or in the presence of various substrates. The concentrations of glycogen, inorganic phosphate, phosphocreatine, adenosine triphosphate, K^+, and Na^+ were studied with glucose as the oxidizable substrate. Formation of lipid peroxides, thought to be connected with oxygen toxicity, was measured by direct chemical analysis, whereas formerly it was estimated indirectly from cerebral peroxidized unsaturated fatty acids (*7*).

Cerebral cortex slices (0.35 mm thick, weighing 60 to 90 mg) from guinea pigs were cut dry with a bow cutter and incubated in Krebs-Ringer glucose saline solution [buffered with tris(hydroxymethyl)aminomethane] (*8*). All incubations were carried out at 37°C in 5 ml

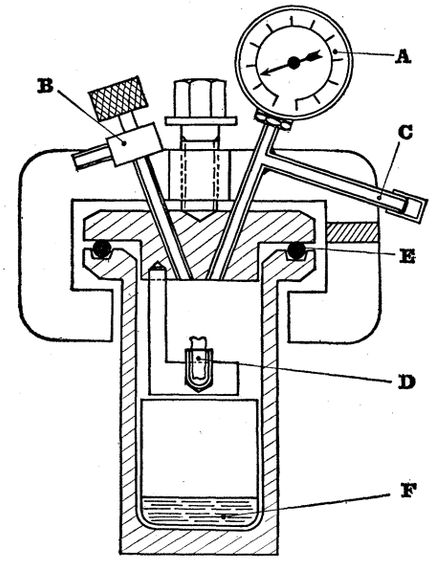

Fig. 1. Sealed cylinder used for the incubation of slices under high oxygen pressure. *A*, Manometer; *B*, valve; *C*, valve of car-tire, for gas entry; *D*, paper saturated with KOH; *E*, rubber rim; *F*, saline (5 ml) in a polythene container. Total volume of the sealed cylinder is 200 ml.

of medium and under an atmosphere of pure O_2. The small, hyperbaric steel chamber (Fig. 1) was shaken 120 times per minute. The substrates used were (20 μmole/ml) D-[^{14}C]glucose (10 m*M*), [2-^{14}C]pyruvate, [1,4-^{14}C]succinate, [1,4-^{14}C]fumarate, γ-amino[1-^{14}C] butyrate (GABA), and L-[1-^{14}C]glutamate. They were sodium salts (Calbiochem, Los Angeles) usually at a concentration of 1.5 μc per 5 ml of saline. The radioactivity was measured in a Beckman 7066 scintillation counter (*9*). After the slices were incubated, they were immediately homogenized in 3 ml of 0.6*M* cold trichloroacetic acid and centrifuged (3000*g*, 10 minutes). Inorganic phosphate and phosphocreatine were separated from the extracts by barium fractionation (*10*). Adenosine triphosphate (ATP) was analyzed separately in trichloroacetic acid extracts after adsorption on activated charcoal and after acid hydrolysis for 10 minutes in 1*N* HCl (*11*). Inulin (1 percent) (British Drug House) was added to measure intracellular space. Noninulin K^+ and Na^+ concentrations were estimated as described (*8*, *12*). Glycogen was precipitated (*13*), and, after hydrolysis, glucose was determined colorimetrically by the specific glucose oxidase method (*14*). After the lipid peroxides were extracted in a mixture of chloroform and ethanol, they were analyzed by thiofluorescein colorimetry (*15*). The [^{14}C]CO_2 evolved at the different pressures is expressed as a percentage of initial radioactivity at 1 atm (Table 1).

The inhibition of oxidation of GABA and glucose, although high at 3 to 6 atm, was significantly less at 10 atm than the inhibition of oxidation of other substrates. After glycogen was incubated at 3 to 10 atm for 60 minutes in the presence of glucose (10 μmole/ml), its concentration (micromoles of glucose per gram of fresh tissue) was reduced below that at 3 atm (1 atm, 1.02 ± 0.18; 3 atm, 0.98 ± 0.2; 6 atm, 0.71 ± 0.12; and 10 atm, 0.37 ± 0.08; mean and S.D. of eight slices). At the same time, the slices resynthesized very little or no phosphocreatine (Fig. 2). Adenosine triphosphate was significantly reduced when the pressure was increased to 10 atm, and inorganic phosphate was markedly enhanced at 6 atm.

The inulin space of tissue (481 ± 45 μl per gram of incubated tissue) was not significantly different between 1 and 10 atm. The K^+ and Na^+ gradients, measured after 60 and 120 minutes under hyperbaric oxygen, continually decreased in the direction of their electrochemical gradients (Table 2). Both these changes were a function of the oxygen pressure and of the time of incubation. These findings are in agreement with the observed changes in total alkaline metal ions previously described (*16*). Under hyperbaric oxygen, inhibition of oxidative, energy-yielding reactions occurs rapidly in vitro, leading to a decrease in the content of energy-rich phosphate bonds which are considered to be directly involved in the maintenance of cell active transport. Also, an increase in apparent intracellular brain Na^+ (or a decrease of K^+) occurs parallel with an enhancement of brain excitability (*17*). Such changes could lead to the convulsions found with hyperbaric oxygen.

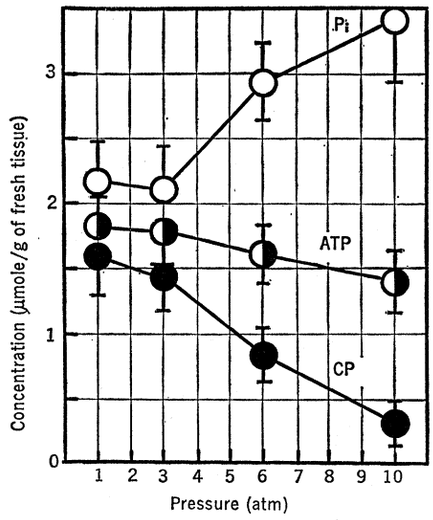

Fig. 2. Concentrations of inorganic phosphate (P_i,) adenosine triphosphate (ATP), and phosphocreatine (CP) in cerebral slices under high oxygen pressure in Krebs-Ringer tris saline solution. Incubation was 60 minutes. The vertical bars represent the standard deviation calculated on eight slices.

After the lipid peroxides were extracted, direct estimation of "true" peroxides ($10^{-6}M$ atom of oxygen per gram of incubated tissue) showed that they increased significantly from 0.550 ± 0.06 ($n = 8$) at 1 atm to 0.866 ± 0.071 ($n = 8$) at 10 atm. These results are consistent with those of Wollman (*18*) and Becker and Galvin (*19*), who have demonstrated that hyperbaric oxygen causes significant elevation in vivo of malonaldehyde-like compounds reacting with thiobarbituric acid; they are also consistent with the correlations noted between clinical symptoms and lipid peroxide concentrations accompanied by acetylcholine esterase suppression (*19*).

Hyperbaric oxygen appears to alter cellular metabolism. However, the specific cellular mechanism of oxygen tox-

Table 1. Effect of hyperbaric oxygen on the evolution of [^{14}C]CO_2 (percentage of radioactivity at 1 atm) in guinea pig brain cortex slices in the presence of different substrates in Krebs-Ringer tris saline (*p*H 7.4) at 37°C after 60 minutes of incubation. The substrate concentration was 20 μmole/ml in all cases except glucose (10 μmole/ml). Results are expressed as the mean ± S.D.; parentheses indicate the number of slices.

Substrate	CO_2 evolution at oxygen pressure		
	3 atm	6 atm	10 atm
Glucose (12)	77.0 ± 4.0	65.5 ± 3.8	48.0 ± 4.0
Pyruvate (8)	86.5 ± 3.6	48.0 ± 2.8	18.0 ± 1.5
Succinate (10)	71.5 ± 3.5	52.0 ± 2.6	30.0 ± 1.8
Fumarate (8)	92.0 ± 3.2	73.5 ± 2.0	42.0 ± 1.9
GABA (8)	87.0 ± 2.8	72.0 ± 3.0	59.0 ± 2.6
L-Glutamate	56.0 ± 2.0	48.0 ± 2.1	33.0 ± 1.6

Table 2. Change in the noninulin K^+ and Na^+ content of guinea pig brain cortex slices under hyperbaric oxygen. The slices were incubated for 30 and 60 minutes in Krebs-Ringer tris saline solution at the pressures indicated. Parentheses indicate the number of slices. Results are expressed as the mean ± S.D.

Pressure (atm)	Concentration in noninulin per gram of incubated tissue			
	K^+ (μeq)		Na^+ (μeq)	
	60 minutes	120 minutes	60 minutes	120 minutes
1	112.0 ± 5.3 (6)	98.6 ± 4.8 (8)	57.0 ± 6.8 (6)	60.9 ± 8.0 (6)
3	90.0 ± 5.0 (8)	73.0 ± 3.8 (8)	75.0 ± 7.2 (8)	107.0 ± 9.5 (8)
6	83.0 ± 4.6 (8)	33.0 ± 2.0 (8)	85.3 ± 6.6 (8)	118.0 ± 10.5 (8)
10	76.0 ± 5.2 (8)	20.5 ± 1.2 (8)	92.6 ± 3.8 (8)	124.0 ± 9.0 (8)

icity is unclear. Free radicals could play an important role (*7*) in that they can inactivate thiol enzymes or coenzymes and damage proteins (*20*). Considerable alterations of permeability of the mitochondrial membrane are correlated with peroxidation of lipoproteins (*21*). Alterations of neuronal membrane permeability might contribute to the effects of oxygen toxicity, especially since active cation transport is now believed to act as a pacemaker for metabolism (*22*).

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19 September 1969; revised 24 November 1969

Wax Esters in Marine Copepods

Abstract. *Two pelagic copepods,* Calanus helgolandicus *and* Gaussia princeps, *contained wax esters with 28 to 44 carbon atoms as major lipid constituents. In laboratory cultures of the former species, changes in nutrition (amount or species of diatoms fed) affected both the amount of total lipid and the composition of the wax esters. Thus, the wax esters serve as a reserve energy store in this organism.*

The calanoid copepods occupy a key position in the marine food chain because they (i) feed directly on phytoplankton, the primary producers in the oceans, (ii) constitute the largest single fraction of the biomass of the zooplankton in most waters, and (iii) are the principal food at some stage in the life of many fish species of nutritional and economic importance to man, such as the herring, sardine, and anchovy (*1, 2*). We have investigated the lipids of several species of these crustaceans (Table 1). The lipids of the large black *Gaussia princeps* from depths of 650 to 900 m contained a high percentage of wax esters, approaching that found in the muscle of some fishes (*3*). The cosmopolitan *Calanus helgolandicus,* collected some distance offshore in plankton tows to 200 m, contained wax esters in lesser proportions (30 to 37 percent), but still as a major lipid type. Two additional species collected at sea off La Jolla were

Table 1. Composition of the lipids of calanoid copepods. *Calanus helgolandicus* wild type a was collected off La Jolla, California, in April 1969; wild type b was collected off La Jolla, California, in June 1969; laboratory grown c was fed *Skeletonema* at 400 μg of carbon per liter; laboratory grown d was fed *Skeletonema* at 600 to 800 μg of carbon per liter. *Gaussia princeps* wild type was collected over Rodriguez Seamount, off Santa Barbara, California, in April 1967. Values are expressed as weight percent.

Fraction	*Calanus helgolandicus*				*Gaussia princeps* wild type
	Wild type		Laboratory grown		
	a	b	c	d	
Hydrocarbons	Trace	3	3	1	Trace
Wax esters*	37	30	25	41	73
Trigylcerides	5	4	3	12	9
Polar lipids†	14	17	10	16	17
Phospholipids‡	44	45	59	28	
Total lipid (% dry weight)	12.4	15	18.6	28	28.9

* Includes any sterol esters present. † Free acids, cholesterol, mono- and diglycerides, and so forth. ‡ Largely lecithin and phosphatidyl ethanolamine determined by thin layer chromatography.

Table 2. Wax esters of *Calanus helgolandicus* under various dietary regimens. Wild type a was collected off La Jolla, California, in April 1969; 200 adult females plus 200 adult males yielded 2.46 mg of lipid; total phytoplankton available to these animals was estimated at between 30 and 150 μg of carbon per liter. Wild type b (250 adult females) was collected off La Jolla, California, in June 1969; available food similar to wild type a. Wild type c is part of collection b that was kept 7 days in the laboratory without food. The laboratory grown was fed *Skeletonema* at the rates indicated (micrograms of carbon per liter); each column represents data from 20 to 105 adult copepods raised in the laboratory from eggs through the nauplii and copepodite stages over a period of 3 to 4 weeks on the specified diet. Values are expressed as weight percent; N.D., not detected.

Chain length*	Wild type			Laboratory grown			
	a	b	c	800	400	200	100
30	2.8	19.8	65.0	21.0	12.0	80.6	89.7
31		trace	4.1			6.7	5.1
32	8.3	4.3	6.6	32.3	30.6	9.1	4.3
34	16.9	10.4	10.3	11.6	16.5	3.6	N.D.
36	23.2	20.4	11.3	18.2	24.1	N.D.	
38	17.9	16.5	6.0	17.9	13.1		
40	9.5	8.3	N.D.	3.7	2.8		
42	8.9	6.6		N.D.	N.D.		
44	7.1	7.3					
Total lipid (% dry weight)	12.4	15.4	7.7	36.6	18.1	15.0	9.3

* Total carbons, alcohol plus acid.