$MgCl_2$; ICDH was best activated by NaCl (0.1–0.4 *M*), and it was best protected by $MgCl_2$. On the other hand, GDH, ME and CS were maximally activated by KCl; at low concentrations $CaCl_2$ and $MgCl_2$ were efficient activators, but they caused a relative inhibition over 0.05 *M*. On the basis of the experimental data described, we suggest that GDH, ME and CS, but not the other enzymes studied here, require for maximal activity the stabilization of hydrophobic bonds by monovalent cations at concentrations higher than 1*M*, the divalent cations being ineffective for this purpose.

It is difficult to ascertain at present the role of the divalent cations in the maintenance of the native structure of halophilic enzymes in vivo. The intracellular concentrations of K^+ and Na^+ are undoubtedly much higher than those of Mg^{++} and Ca^{++}, but the concentrations of the latter cations required for activation are considerably lower. Moreover, preliminary experiments, not shown here, demonstrate that $MgCl_2$ or $CaCl_2$, at 0.1 *M*, can interact with halophilic enzymes even in the presence of 2.3 *M* KCl, causing in general a relative inhibition. The recent report by LANYI and SILVERMAN[10] on the state of the intracellular cations in *H. cutirubrum* may be relevant to this problem. They showed that, whereas K^+ seems to be free inside the cell, Mg^{++} is bound, probably to both the membrane lipids and the acidic proteins[11] present in extreme halophiles. The possibility cannot be excluded, then, that divalent cations, mainly Mg^{++}, might participate in the activation and stabilization of halophilic enzymes in vivo.

Table II. Stabilization of halophilic enzymes by divalent and monovalent cation salts

	GDH	MDH	ME	ICDH	GluDH	AAT	CS
Basal	0.1	0.4	3.7	< 0.1	< 0.1	5	<0.1
$CaCl_2$	1140	360	27	5.6	8	50	0.5
$MgCl_2$	2460	220	40	66	9	125	0.2
NaCl	960	450	310	10	5400	350	145
KCl	3240	75	180	3.3	132	120	53

The experiments were performed at 30 °C, as described in ref.[14]. The basal NaCl concentration was 0.1*M*, except for CS (0.2*M*). The protein concentrations in the preincubation mixtures (μg/ml) were, respectively: GDH, 158; MDH, 54; ME, 336; ICDH, 186; GluDH, 52; AAT, 83; and CS, 20. The salt concentrations tested as protectors were 0.4*M* Me^+ or Me^{++} (AAT), or 1*M* Me^+ or 0.1*M* Me^{++} for the other enzymes. The half-life values (in min) obtained from semilogarithmic plots of remaining activity *vs* time of preincubation are given as a measure of the stabilizing effect of the salts.

Resumen. Siete enzimas parcialmente purificadas de la bacteria halófila extrema *Halobacterium cutirubrum* fueron activadas, y la mayoría de ellas parcialmente estabilizadas, por concentraciones de Ca^{++} o Mg^{++} hasta 0.4 *M*. En algunos casos las sales de catión divalente fueron más eficaces que el ClNa o el ClK. Se discuten los resultados en relación con la posible participación del escudado de cargas y del refuerzo de uniones hidrofóbicas en el mantenimiento de la estructura nativa, y con el posible papel del Mg^{++} in vivo en la activación y estabilización de enzimas halofílicas.

A. I. HIGA, M. C. VIDAL and J. J. CAZZULO[15]

Departamento de Bioquímica, Facultad de Ciencias Bioquímicas, Universidad Nacional de Rosario, Suipacha 531, Rosario (República Argentina), 30 August 1973.

[10] J. K. LANYI and M. P. SILVERMAN, Can. J. Microbiol. *18*, 993 (1972).
[11] R. REISTAD, Arch. Mikrobiol. *71*, 353 (1970).
[12] A. I. HIGA and J. J. CAZZULO, Experientia, *29*, 1081 (1973).
[13] J. S. HUBBARD and A. B. MILLER, J. Bact. *99*, 161 (1969).
[14] J. J. CAZZULO and M. C. VIDAL, J. Bact. *109*, 437 (1972).
[15] This work was supported by a grant from the Consejo Nacional de Investigaciones Cientificas y Técnicas (Argentina); JJC is a member of the Research Career of the same institution. MCV is a member of the Research Career of the Consejo de Investigaciones of the Universidad Nacional de Rosario, and AIH is the recipient of a fellowship from the latter institution.

Lipid Metabolism in Suckling Rats with Fatty Liver Induced by Hypoxia

Previous studies have shown a fatty liver degeneration in baby rats born and kept at 3990 m above sea level or submitted to a simulated altitude of about 4600 m since 1-day-old. A depression of oxygen consumption was observed in these chronic hypoxic suckling rats[1] and the suggestion made that it could be due to decreased tissue oxidation. This would impair lipid utilization, which could result in fatty liver degeneration. To further investigate this hypothesis, plasma and liver total lipids and blood β-hydroxybutyrate and acetoacetate levels were measured in normal and in chronically hypoxic rats which were either sacrificed immediately after removal from the hypobaric chamber or kept at sea level pressure for 8 to 48 h before being sacrificed.

Material and methods. Litters of Sprague-Dawley rats were reduced to 10 rats at 1 day of age. Some litters were kept at sea level pressure as controls. The others were placed with the mother in a hypobaric chamber maintained at a simulated altitude of about 4600 m. The chamber was opened every other day to clean, replace food and water, and weight the litter.

The hypoxic and control rats were decapitated and the blood was collected in a heparinized container. The plasma sample was obtained by centrifuging the blood in microhematocrit tubes. Since the amount of blood obtained from each rat was too small to permit measurement of both acetoacetate and β-hydroxybutyrate, a different animal was used for each measurement. Total lipids in plasma and in liver were measured in each rat. Acetoacetate and β-hydroxybutyrate levels in the blood were determined using the enzymatic micromethod described by WILDENHOFF[2]. The standards used in the method were sodium β-hydroxybutyrate, which was recrystallized twice, and lithium acetoacetate, which was prepared according to the method described by HALL[3]. Total plasma lipids were measured by using the E. Merck Total Lipid test kit. Total lipids in liver were determined by a

[1] H. CHIODI, Am. J. Physiol. *218*, 92 (1970).
[2] K. E. WILDENHOFF, Scand. J. clin. Lab. Invest. *25*, 171 (1970).
[3] L. M. HALL, Analyt. Biochem. *3*, 75 (1962).

Table I. Blood β-hydroxybutyrate, plasma total lipids and liver total lipids in suckling rats kept at a simulated altitude of about 4600 m since 1-day-old

Condition	No. of animals	Age in days at death	Time outside chamber before death (h)	Body wt. (g)	Blood β-hydroxybutyrate (μmole/100 ml)	Plasma total lipids (mg/100 ml)	Liver total lipids (% wet wt.)	Hct (%)
Chronic hypoxia	10	12	0	14.3 ± 0.4[a]	121.0 ± 13.8	1660 ± 152	10.6 ± 0.9	41.6 ± 0.8
Chronic hypoxia	10	11	8	14.9 ± 0.4	143.3 ± 11.4	1103 ± 50	7.4 ± 0.7	52.6 ± 0.8
Chronic hypoxia	10	12	24	17.3 ± 0.7	168.8 ± 15.8	1029 ± 84	5.0 ± 0.9	46.9 ± 1.0
Chronic hypoxia	3	3	48	18.7 ± 0.9	90.9 ± 17.3	970 ± 138	7.4 ± 0.6	56.4 ± 0.4
Controls	10	12	0	21.3 ± 0.3	131.4 ± 7.4[b]	531 ± 13	3.4 ± 0.08	35.1 ± 0.3

[a] Standard error of the mean. [b] 9 animals.

method previously described[4]. All of the rats had stomachs full of milk when they were sacrificed. The presence of exogenous lipids seemed undesirable in the present study, but the rapid mobilization of lipids from the liver and the increased production of ketone bodies together with a progressive dehydration experienced by baby rats when fasted for even a few hours induced us to use control and experimental animals under similar fed conditions.

Results. As shown in Tables I and II, the total lipids in plasma and liver in chronically hypoxic rats are greater than those in control rats of the same age ($p < 0.001$). A 12-day-old control litter (Table I) is used for control values.

In hypoxic rats returned to sea level pressure for 8 h before collecting samples, the total liver lipids were significantly lower than in hypoxic rats sacrificed immediately after removal from the chamber ($p < 0.02$), but still significantly higher than in control rats ($p < 0.001$). Liver lipids in hypoxic rats returned to sea level for 24 h were not significantly different from the controls ($p > 0.10$). In the 48-hour litter, 4 died in the chamber, 3 died during the first 24 h at sea level pressure and 3 survived 48 h outside the chamber. It seems that in the 3 latter animals the liver functions were already impaired to such a degree that recovery under the influence of normal oxygen pressure was no longer possible. This could explain the higher level of liver lipids and the low β-hydroxybutyrate values in these rats compared with those found in the hypoxic rats kept 24 h at sea level pressure before being sacrificed.

In the hypoxic rats, β-hydroxybutyrate values were not significantly different from those of the controls (Table I), but when the hypoxic rats were kept 24 h at sea level pressure before being sacrificed, β-hydroxybutyrate values increased significantly ($p < 0.05$) over those found in hypoxic rats which were sacrificed immediately after removal from the chamber.

In the hypoxic rats, acetoacetate mean values were not significantly different from values found in the control rats of about the same age ($p > 0.10$; Table II). The β-hydroxybutyrate: acetoacetate ratio decreased from 3.4 in the controls to 2.7. in the hypoxic suckling rats, or a 21% decrease.

Discussion. Confirming previous findings[4], chronic hypoxia due to a simulated altitude of 4600 m induced hyperlipemia and fatty liver in suckling rats, which were maintained under hypoxic stress for 10–11 days, beginning at the age of 1 day.

No significant differences in β-hydroxybutyrate or acetoacetate levels in the blood were observed between hypoxic and control rats of the same age. When the hypoxic rats were returned to sea level pressure for 8, 24 and 48 h prior to sacrifice, the levels of total lipids in plasma and liver decreased significantly. The total liver lipids fell to normal values in the litter kept for 24 h at sea level pressure. This rapid decrease in lipid content of the hepatic cells may be explained on the basis of an increased mobilization of lipids toward extra hepatic tissues such as adipose tissue and/or an increase in oxidation of liver lipids previously hindered by the chronic hypoxic state.

In normal conditions during the suckling period, rat liver has an increased capacity for fatty acid degradation and ketone body formation[5]. In our hypoxic rats, blood ketone body values remained within the normal range. It seems that in the presence of a low level of liver glycogen, an abnormally high plasma, and liver lipid levels fail to

[4] H. CHIODI and R. BASS, Fedn. Proc. *28*, 1080 (1969).
[5] E. BAILEY and E. A. LOCKWOOD, Biochem. J. *124*, 7 (1971).

Table II. Blood acetoacetate, plasma total lipids and liver total lipids in suckling rats kept at a simulated altitude of about 4600 m since 1-day-old

Condition	No. of animals	Age (days)	Body wt. (g)	Blood acetoacetate (μmole/100 ml)	Plasma total lipids (mg/100 ml)	Liver total lipids (% wet wt.)	Hct (%)
Chronic hypoxia	10	11	13.5 ± 0.4[a]	43.7 ± 2.9	1394 ± 97	12.8 ± 0.6	44.5 ± 0.6
Controls	10	11	20.9 ± 0.4	37.6 ± 2.5	562 ± 17	3.6 ± 0.04	34.2 ± 0.3

[a] Standard error of the mean.

stimulate ketogenesis[6], but the formation of acetoacetate is favored over that of β-hydroxybutyrate. The decrease of the β-hydroxybutyrate: acetoacetate ratio in the blood might be explained on the basis of the low levels of hepatic glycogen found in our hypoxic rats[7].

There is a significant increase in β-hydroxybutyrate blood levels in hypoxic rats when returned for several hours at sea level before being killed. This increase could be due to the sudden removal of the depressant effect of hypoxia on lipid oxidation. The rapid increase in total oxygen consumption found in hypoxic baby rats when switched from a low to a normal oxygen environment[1] could be due to the sudden increase in the oxidation rate.

Zusammenfassung. Versuche über die Anreicherung von Lipiden in der Leber und im Serum hypoxisch gehaltener Ratten, die zeigen, dass auch bei Sauerstoff-Mangelzuständen des Menschen die Lipidanreicherung zur Verschlechterung der Situation beitragen könnte.

H. CHIODI[8] and S. WHITMORE[9]

Department of Ergonomics, University of California, Santa Barbara (California 93106, USA) and Cardiovascular Research Institute, University of California, San Francisco (California 94122, USA),
2 October 1973.

[6] A. WHITE, P. HANDLER and E. L. SMITH, *Principles of Biochemistry* (McGraw-Hill Book Co., New York, 1968).
[7] H. CHIODI and Z. V. BACA, Revta Soc. Argent. Biol. *39*, 69 (1963).
[8] Present address: Saint Joseph Medical Center, Pulmonary Function Department, 501 South Buena Vista, Burbank (California 91505, USA).
[9] Program Project Grant No. H. E. 06285.

H_2S als Schwefelquelle bei *Lemna minor* L.: Einfluss auf das Wachstum, den Schwefelgehalt und die Sulfataufnahme

Höhere Pflanzen sind in der Lage, neben Sulfat eine ganze Reihe von Verbindungen zur Deckung ihres Schwefelbedarfs heranzuziehen[1,2]. In diesem Zusammenhang fehlen im Gegensatz zu Schwefeldioxid eingehende Untersuchungen über Schwefelwasserstoff, obschon grüne Pflanzen ganz allgemein an Standorten wie z.B. sulfidhaltigen Böden[3], flachen Seen und Teichen[4], vulkanischen Gebieten[5], Umgebungen von Schwefelquellen[6] und Gebieten in der Nähe von emittierenden Industrie- und Gewerbebetrieben[7] damit in Berührung kommen. Lemnaceen im speziellen wachsen oft auf Teichen, wo sich Schwefelwasserstoff nachweisen lässt[8]. Es stellt sich hier natürlich in erster Linie die Frage nach der Resistenz gegen die als Zellgift allgemein bekannte Verbindung und deren Einfluss auf das Wachstum. Daneben interessiert vor allem die Beeinflussung des Schwefelhaushalts.

Material und Methoden. Lemna minor L., Stamm Nr. 6580 der Lemnaceensammlung von LANDOLT[9], wird auf 30 ml Nährlösung (H8[10]) in 150 ml Erlenmeyerkolben mit Wattestopfen auf weisser Unterlage bei 3000 Lux (Dauerbeleuchtung mit Fluoreszenzröhren Philips TL 33), einer Temperatur von 25 ± 1 °C und einer relativen Luftfeuchtigkeit von 65–80% aseptisch kultiviert. Zur Ermittlung der CO_2-Fixierungsrate diente das von ERISMANN[11] entwickelte System für Gaswechselmessungen, in welchem die CO_2-Konzentration mit einem Uras gemessen wird. Für die vorliegende Arbeit wurde es mit einem H_2S-Analysator (Jonoflux, Hartmann und Braun, Frankfurt), ergänzt.

Die Wachstumsversuche wurden auf einer speziell für Lemnaceen gebauten Wachstumsanlage[12] durchgeführt. Die spezifische Wachstumsrate μm wurde durch Auszählen der Glieder auf vergrösserten photographischen Negativen ermittelt:

$$\mu m = \frac{ln\ \text{Gliederzahl}\ t_2 - ln\ \text{Gliederzahl}\ t_1}{t_2 - t_1}$$

(t in Tagen).

Zur Dosierung des H_2S diente ein Diffusionsverfahren durch einen Gummischlauch[12]. Die Sulfat- und Gesamt-

[1] M. D. THOMAS in *Encyclopedia of Plant Physiology* (Ed. W. RUHLAND; Springer, Berlin 1958), vol. 9, p. 37.
[2] R. J. ELLIS, Planta *88*, 34 (1969).
[3] H. WALTER, *Vegetation der Erde* (Fischer, Jena 1968), vol. 3.
[4] V. CZURDA, Zbl. Bakt. *103*, 285 (1941).
[5] K. GARBER, *Luftverunreinigungen und ihre Wirkungen* (Bornträger, Berlin-Nikolassee 1967).
[6] A. SCHATZ, Bryologist *58*, 113 (1955).
[7] H. C. MÜLLER, Arb. Landw. K. Sachsen *13*, 93 (1913).
[8] K. W. KUCHAR, Arch. Hydrobiol. *49*, 329 (1954).
[9] E. LANDOLT, Ber. Schweiz. bot. Ges. *67*, 271 (1957).
[10] K. H. ERISMANN und A. FINGER, Ber. Schweiz. bot. Ges. *78*, 5 (1968).
[11] K. H. ERISMANN, Habilitation Universität Bern (1969).
[12] K. H. ERISMANN und CHR. BRUNOLD, Ber. Schweiz. bot. Ges., im Druck.

Spezifische Wachstumsrate, mittleres Trockengewicht pro Glied und Schwefelgehalt von *Lemna minor* bei Kultivierung mit verschiedenen Schwefelquellen

Schwefelquelle	Spezifische Wachstumsrate[a] (μm)	Mittleres Trockengewicht pro Glied[b] (mg)	Totalschwefel[b] (mg/g Trockengewicht)	Sulfatschwefel[b] (mg/g Trockengewicht)	Sulfidschwefel[b] (mg/g Trockengewicht)
4×10^{-4} *M* SO_4^{2-}	0,457 ± 0,011	0,091 ± 0,004	2,42 ± 0,12	0,50 ± 0,02	–
6 ppm H_2S	0,379 ± 0,014	0,081 ± 0,004	6,54 ± 0,43	4,50 ± 0,17	0,032 ± 0,002
4×10^{4} *M* SO_4^{2-} 6 ppm H_2S	0,392 ± 0,010	0,084 ± 0,003	6,7 ± 0,41	4,58 ± 0,19	0,029 ± 0,002

[a] 5 Bestimmungen vom 7.–13., 13.–17., 17.–24., 24.–30. und 30.–36. Kultivierungstag. [b] 4 Bestimmungen am 10., 18., 25. und 36. Kultivierungstag.