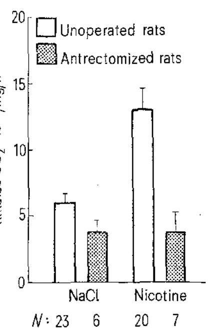
5×10^{-4} *M* glutathione and 4×10^{-4} *M* 1-^{14}C-L-histidine (1.3 mC/m*M*) added in this order. The mixture was gassed with nitrogen for 5 min at 0 °C. After 1 h at 37 °C the reaction was terminated by the addition of 0.5 ml 10% trichloroacetic acid and the evolved $^{14}CO_2$ was trapped during 30 min at 37 °C on a filter paper strip, previously immersed in Protosol® and placed in a central well in the reaction vessel. The radioactivity was then determined in a liquid scintillation spectrometer. The results were corrected for non-enzymatic decarboxylation by incubating identical samples with 1-^{14}C-D-histidine instead of 1-^{14}C-L-histidine. Duplicate assays were run in all experiments.



The nicotine-induced activation of rat stomach histidine decarboxylase is prevented by antrectomy. Nicotine: 0.2 mg/kg. Controls received 0.9% saline. The rats were killed 2 h after the injection. Enzyme activities are expressed as nmoles CO_2 produced per mg tissue and hour. *N* denotes the number of rats in each group.

Single injections of 0.2 or 1 mg/kg of nicotine caused activation of gastric histidine decarboxylase in normal, fasted rats, whereas a dose of 5 mg/kg failed to raise the enzyme activity (Table I). The enzyme activity seemed to reach a peak $1^1/_2$–2 h after administration of nicotine (Table II). Antrectomy prevented the nicotine-induced enzyme activation (Figure). It may thus be suggested that nicotine activates gastric histidine decarboxylase through the release of gastrin. The results do not support the alternative explanation that nicotine has a direct effect on the enzyme-containing cells.

Zusammenfassung. Nikotin steigert die gastrische Histidindekarboxylasaktivität in normalen, nüchternen, nicht aber in antrektomierten Ratten. Dieses weist darauf hin, dass die Aktivierung durch nikotininduzierte Freisetzung von antralem Gastrin verursacht wird.

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The Bohr Effect and the Red Cell 2-3 DPG and Hb Content in Sherpas and Europeans at Low and at High Altitude

In a previous paper[1] we have reported that the Bohr effect of individuals of Amerindian origin living in the highlands of Peru was significantly higher than that of Europeans living at the same altitude and we have interpreted this difference as the effect of an evolutionary adaptation to life at low oxygen pressure.

The aim of the present research was 1. to ascertain whether in a different population, living at an altitude comparable with that of the Peruvian population, the same adaptation has occurred; 2. to collect more data on Europeans in order to know if physiological phenomena of adaptation resulting into an increase of the Bohr effect can also occur in Caucasian individuals.

Materials and methods. Four types of individuals were examined: 1. Sherpas living at high altitude; 2. Sherpas living at low altitude; 3. Europeans at high altitude and 4. Europeans at low altitude.

The research has been carried out in Nepal in October 1970. The high altitude samples were taken at 4950 metres above the sea level, in the region of Solo Khumbu, from 24 porters of the expedition and 12 members of an Italian alpinistic team. The bleedings were done after staying for three to four days at that altitude.

The low altitude Sherpa samples were obtained from a group of Sherpas of the region of Helambu, (about 3500 metres above the sea level) but living in Kathmandu (1200 metres above sea level)[2]. The European samples at low level were obtained from the same alpinistic team in Italy before the departure of the expedition.

The oxygen dissociation curves were determined in Rome by a previously described spectrophotometric technique[3] on airshipped hemolysates prepared and reduced with nitrogen in the field (previous experiments have shown to us that the oxygen affinities of reduced hemolysates is stable for months).

The haemoglobin content per ml of whole blood was determined by the cyanmethaemoglobin method[4]. The red cell 2–3 DPG was determined according to BARTLETT[5] on hemolysates whose 2–3 DPG was stabilized by boiling the specimens diluted in distilled water. All the hemolysates were also examined by starch gel electrophoresis according to GOLDBERG[6] and only normal haemoglobin ($A + A_2$) were detected.

Results and discussion. The results are shown in detail in Tables I and III and their comparisons are shown in Table IV.

Table I shows the data concerning the Europeans at low and high altitude. The Bohr effect and mean Hb content of the 2 samples of Europeans are not significantly different from each other (see Table IV). However, some interesting intracouple (subjects No. 5, 9 and 12) difference is apparent (the intracouple data refer to those subjects who were tested both at low and at high altitude).

[1] G. MORPURGO, L. BERNINI, P. BATTAGLIA, A. M. PAOLUCCI and G. MODIANO, Nature, Lond. *227*, 387 (1970).

[2] The Sherpas of the Helambu region are probably of the same racial stock as those of the Solo Khumbu region. The precise history of the Sherpa people is unknown, but it seems that they derive from a Tibetan population which crossed the Himalaja a few centuries ago.

[3] T. LEGGIO and G. MORPURGO, Annali Ist. sup. Sanità *4*, 373 (1968).

[4] E. J. VAN KAMPEN and W. G. ZIJLSTRA, Clin. chim. Acta *6*, 538 (1961).

[5] G. R. BARTLETT, J. biol. Chem. *234*, 459 (1959).

[6] C. A. GOLDBERG, Clin. Chem. *4*, 485 (1958).

Table I. The distributions of the P_{50} at pH 7.4 and 6.8, of the Bohr effect, of the Hb and 2–3 DPG red cell contents in Europeans bled at low and at high altitude

a) Europeans at the sea level

Specimens	P_{50}		Bohr effect (b-a)	g of Hb/100 ml of blood	2–3 DPG μmoles/1 ml of blood
	pH 7.4 (a)	pH 6.8 (b)			
1	21	30	9	16.37	2.35
2	22	27	5	17.11	2.20
3	21	26	5	16.37	1.90
4	22	28	6	17.66	1.90
5	25	30	5	16.92	2.25
6	23	28	5	16.37	2.25
7	23	30	7	16.37	2.35
8	23	30	7	17.29	2.20
9	22	30	8	17.29	2.20
10	21	26	5	16.56	1.90
11	22	30	8	17.84	1.85
12	22	30	8	16.74	2.35
13	22	28	6	16.37	2.40
Mean	22.23	28.69	6.46	16.87	2.162
Coefficient of variation = m.s.d./mean × 100	4.90	5.58	22.4	3.14	29.4

b) Europeans at high altitude (4950 m)

Specimens	P_{50}		Bohr effect (b-a)	g of Hb/100 ml of blood	2–3 DPG μmoles/1 ml of blood
	pH 7.1 (a)	pH 6.8 (b)			
1	21	30	9	15.01	3.25
2	21	27	6	15.12	3.38
3	20	27	7	16.19	3.64
7	19	25	6	15.05	3.90
9	25	35	10	16.37	4.16
10	21	27	6	15.31	3.64
11	21	31	10	18.21	2.60
12	24	29	5	14.31	4.55
13	—	—	—	15.64	2.60
14	21	33	12	16.56	4.68
15	23	28	5	18.40	3.12
16	23	27	4	16.92	3.25
17	21	28	7	16.19	2.99
Mean	21.67	28.92	7.25	16.10	3.520
Coefficient of variation = m.s.d./mean × 100	7.94	9.92	33.8	7.58	18.9

In two cases (9 and 14) we have found in the sample taken at high altitude an exceptionally high Bohr effect. However, it became normalized (see Table II) in the solutions obtained by filtering the hemolysates through G 25 Sephadex columns equilibrated with the same buffers, a finding which strongly suggests that small molecules present in these two hemolysates were responsible for their exceptionally high Bohr effect. The same subjects were bled again in Italy 2 months after the end of the expedition. The Bohr effects turned out to be intermediate between those observed in Nepal, and those of the solutions obtained from Sephadex filtration (see Table II). These results can be tentatively interpreted as an indication that in some Europeans an adaptative physiological change of the Bohr effect occurs in response to the lowering of oxygen pressure. However, it is advisable to be cautious while interpreting these data in view of the recent finding that a strong change of the Bohr effect, apparently non adaptive, can occur in a pathological condition, i.e. in diabetes[7].

A really puzzling finding is that the shift towards the right of both the oxygen dissociation curves which we[1] and other authors[8] have also found in Peru, was not apparent for either the Europeans or the Nepalese in the present case (the same method was used for examining the Peruvian and the Nepales samples).

[7] P. Battaglia, G. Morpurgo and S. Passi, Experientia *27*, 321 (1971).

[8] C. Lenfant, J. Torrance, E. English, C. A. Finch, C. Reynafarje, J. Ramos and J. Faura, J. clin. Invest. *47*, 12 (1968).

Table II. The P_{50} at pH 7.4 and 6.8 and the Bohr effect in different conditions

	The solutions which have been examined	Subject No. 9			Subject No. 14		
		P_{50}		Bohr effect	P_{50}		Bohr effect
		pH 7.4	pH 6.8		pH 7.4	pH 6.8	
a) [a]	Unfiltered hemolysate from blood obtained at the sea level	22	30	8	—	—	—
b) [a]	Unfiltered hemolysate from blood obtained at 4950 m of altitude	25	35	10	21	33	12
c)	The same hemolysate as in b) after having been filtered through a G 25 Sephadex column	19	24	5	19	25	6
d)	Unfiltered hemolysate from blood obtained at the sea level 2 months after the end of the expedition	18	26	7	21	30	9

[a] The data of these 2 raws are those reported in Table I.

Table III. The distributions of the P_{50} at pH 7.4 and 6.8, of the Bohr effect, of the Hb and 2–3 DPG red cell contents in Nepalese bled at low and at high altitude

a) Nepaleses at low altitude (1200 m)

Specimens	P_{50}		Bohr effect (b-a)	g of Hb/100 ml of blood	2–3 DPG µmoles/1 ml of blood
	pH 7.4 (a)	pH 6.8 (b)			
201	—	—	—	12.14	2.99
202	22	29	7	17.07	2.99
203	22	27	5	9.49	3.25
204	20	26	6	12.03	3.12
205	23	28	5	13.98	3.26
206	24	28	4	16.48	3.25
207	20	26	6	15.93	3.12
208	23	29	6	13.65	2.60
209	22	28	6	14.60	3.05
210	21	27	6	14.20	2.34
212	21	26	5	14.83	3.12
213	18	26	8	15.83	2.60
214	20	26	6	14.90	2.60
215	25	30	5	13.10	3.25
Mean	21.62	27.38	5.77	14.16	2.967
Coefficient of variation = m.s.d./mean × 100	8.74	5.08	17.5	14.3	10.2

The mean red cell 2–3 DPG content (expressed per ml of blood) turned out to be higher in the subjects bled at high altitude (see Table IV) in agreement with previous data[8]. Changes in the Bohr effect are not, however, correlated with the 2–3 DPG content because the variations of organic phosphate concentrations required for modifying the Bohr effect largely exceed the physiological range, if the hemoglobin is in 0.1 *M* phosphate buffer[9], as in the experiments here discussed.

The oxygen dissociation curves of the 2 groups of Nepalese (Table III) are identical to each other at both the pH's. The comparisons between the Caucasian and the Nepalese show (Table IV): 1. The means of the P_{50} at pH 7.4 and pH 6.8 and of the Bohr effect are not significantly different. 2. The Europeans at high altitude showed an increased variance for all the parameters here studied, in contrast with the Sherpa subjects whose variability at high altitude is high only for the 2–3 DPG. If this high variation observed in the Europeans is caused by the environmental stress this fact could mean that they are more affected at the haematological level by the life at high altitude, possibly because they are less fitted to life in hypoxia than the Sherpas. 3. The 2–3 DPG content of the Sherpas at high altitude does not differ significantly from that of the Europeans at the same altitude[10].

[9] R. BENESCH and R. E. BENESCH, Nature, Lond. *221*, 618 (1969).

[10] Acknowledgments. We wish to thank Mr. G. TENTI, the head of the alpinistic team, for his invaluable help. The expedition was sponsored by grants from the International Biological Program (IBP), Italian Committee of the Consiglio Nazionale delle Ricerche, Rome; from the WHO, from the Istituto Superiore di Sanità, Rome, and from the Club Alpino Italiano.

Table III. The distributions of the P_{50} at pH 7.4 and 6.8, of the Bohr effect, of the Hb and 2–3 DPG red cell contents in Nepalese bled at low and at high altitude

b) Nepaleses at high altitude (4950 m)

Specimens	P_{50} pH 7.4 (a)	P_{50} pH 6.8 (b)	Bohr effect (b-a)	g of Hb/100 ml of blood	2–3 DPG μmoles/1 ml of blood
109	22	31	9	16.37	3.25
110	—	—	—	19.09	4.16
111	21	27	6	16.56	3.77
112	22	27	5	15.82	4.55
114	21	27	6	17.03	3.64
115	—	—	—	16.74	4.16
117	—	—	—	16.78	3.25
118	19	26	7	17.11	3.64
121	23	28	5	17.33	4.55
122	20	25	5	16.48	4.16
123	—	—	—	14.31	3.25
124	24	30	6	18.69	3.25
128	19	25	6	18.32	2.79
129	22	26	4	12.36	2.21
130	—	—	—	16.33	4.55
131	19	26	7	17.11	3.64
132	—	—	—	16.85	3.25
134	23	31	8	18.43	3.25.
Mean	21.25	27.42	6.17	16.76	3.629
Coefficient of variation = m.s.d./mean × 100	8.05	7.84	22.7	9.37	17.7

Table IV. The most relevant comparisons concerning the parameters under study

a) the means

	P_{50} pH 7.4		P_{50} pH 6.8		Bohr effect		g Hb/100 ml of blood		2–3 DPG μmoles/1 ml of blood	
Altitude	low	high	low	high	low	high	low	high	low	high
Europeans	22.23	21.67	28.69[b]	28.92	6.46	7.25	16.87[b,d]	16.10[b]	2.16[d]	3.52[d]
Sherpas	21.62	21.25	27.38[b]	27.42	5.77	6.17	14.16[d]	16.76[d]	2.97[d,c]	3.63[c]

b) the variances

Altitude	low	high	low	high	low	high	low	high	low	high
Europeans	1.19[b]	2.97[b]	2.56[b]	8.27[b]	2.10[b]	6.02[b]	0.28[c,d]	1.50[c]	0.041[a,d]	0.441[d]
Sherpas	3.59[b]	2.93	1.92	4.63	1.03	1.97[b]	4.06[d]	2.45	0.092[a,c]	0.411[c]

Levels of significances of the comparisons: [a] $P \leqq 0.1$; [b] $P \leqq 0.05$; [c] $P < 0.01$; [d] $P \ll 0.001$; the probabilities have been reported only for the comparisons with statistically significant (or almost so) differences.

Riassunto. Europei e Sherpas hanno mostrato, dopo una permanenza di 3–4 giorni ad una altitudine di circa 5000 metri, normali P_{50} (a pH 7.4 ed a pH 6.8) ed effetto Bohr (molto variabile negli Europei) ed elevato contenuto ematico di 2–3 DPG.

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