those reported by Bunn[8] at 20 °C. The values at this temperature are very similar, although a more pronounced difference between the oxygen affinity of Hb A and Hb C is observed. This difference is more markedly increased at 37 °C. The P_{50} detected at 37 °C for Hb A and Hb B are, on the other hand, in good agreement with those derived from data obtained by Bauer[7] (who did not analyze Hb C). Other investigators, who examined the oxygen affinity of Hb C, as well as that of Hb A and Hb B at 37 °C, did not find a significant difference between Hb C and Hb A in whole blood and in hemolysates[9], although in a previous paper they reported a shift of the affinity curve to the left for whole blood containing Hb C[10]. The discrepancy between these results and ours can be ascribed to the different experimental conditions, for example the method of purification of the hemoglobins, the composition of the buffer, the presence of CO_2, and the method used to determine the oxygen saturation.

A larger Bohr effect of Hb C compared to Hb A and Hb B has already been reported, but determined in presence of CO_2[9] (our experiments were performed in absence of CO_2). The findings that a hemoglobin with increased oxygen affinity is produced when hypoxia is of such a degree to put in danger the life of the animal is apparently intriguing. In fact, man and other mammals (dog, horse, pig) which possess hemoglobins with intrinsically high oxygen affinity[8] adapt to hypoxia by lowering the oxygen affinity through the increase of intracellular 2,3-DPG. On the other hand, it is possible that sheep, which have hemoglobins with intrinsically low oxygen affinity that do not bind 2,3-DPG[8], could benefit from the production of a hemoglobin with a raised oxygen affinity and a large Bohr effect. Even in cats, which also have a hemoglobin with low oxygen affinity, phenylhydrazine-induced anemia results in an increase of hemoglobin components with high oxygen affinity[11]. A better resistance to extreme hypoxia was reported in mice, when the oxygen affinity of the hemoglobin was artificially increased[12].

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Improved response to heat after moderate physical training in man[1]

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Summary. 7 young men marched for 6 days (35 km daily) in a cool climate at about 35% of their $\dot{V}O_2max$. Sweat output was measured at rest in a climatic chamber during a controlled hyperthermia test performed before and after the trial. 4 subjects served as controls. The main finding of the study was that sweat output increased 17.3% ± 1.5 SEM on the post-trial test, without any change in $\dot{V}O_2max$. It is concluded that moderate physical training can improve heat responses in resting man.

Since Wyndham's report in 1951[3], acclimatization to heat has been described as occurring in man working in hot environments[4,5]. Heat acclimatization has also been achieved both by repeated passive heating sessions in resting man[6,7] and by physical exercise performed in a cool climate, followed by increased maximal aerobic consumption ($\dot{V}O_2max$)[8–13]. Such results were discussed by Hale[14] and Glaser and Shephard[15] as intervening factors in 'cross adaptation'. However, the specific role of physical exercise in the processes of response to heat has not yet been clarified, since the procedures to test the thermoregulatory response to heat in most of these studies have associated work and heat exposure, and the exercises performed always led to increased physical fitness. Our aim was to eliminate this latter factor by using a method involving a moderate repetitive physical exercise in a cool environment which did not result in a change in physical fitness, and by testing the thermolytic responses to heat in the subject at rest.

Methods. a) The subjects, 12 male Caucasian volunteers, aged 20, were separated into 2 groups. Group 1 consisted of 8 subjects who participated in a field trial in cool weather conditions (ambient temperature = 0–7 °C). This trial consisted of a 6-day walk at 5.6 km · h^{-1} on a flat terrain at 300 m altitude, during 7–8 h a day. The subjects wore standard army clothing and carried a backpack suitably weighted to allow an energy expenditure of approximately 35% of each one's maximal oxygen consumption ($\dot{V}O_2max$ ranging from 48.3 to 62.4 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$)[16]. Group 2, which consisted of 4 sedentary control subjects, performed daily routines throughout the experimental period.

b) The thermal test consisted of a controlled hyperthermia test performed in a climatic chamber[6], the subjects lying nude on a string bed allowing sweating and evaporation. In order to reach steady body temperatures they spent their first 90 min in a thermoneutral environment. The ambient temperature was then raised to reach the target internal temperature (tympanic temperature = 38 °C), which was maintained for 1 h by ambient air humidity adjustments. The sweat loss during the hour of steady hyperthermia was measured by weighing the subject continuously.

c) Experimental procedure: in group 1, the thermoregulatory responses to heat were examined 3 weeks before (pre) and immediately after (post) the field trial (making an

Table 1. Climatic characteristics of the chamber and sweat output during pre- and post-trial controlled hyperthermia tests

Subjects	Pre-trial test				Post-trial test				Variations
	T_{db}	T_{wb}	Rh	E	T_{db}	T_{wb}	Rh	E	ΔE
S_1	45.0	35.0	53	272	47.0	36.0	50	319	+17.3
S_2	44.5	37.0	62	322	46.0	37.0	58	369	+14.6
S_3*	45.0	36.0	55	320	47.5	37.5	54	342	+ 6.8
S_4	44.0	36.0	59	359	48.5	38.5	56	442	+23.2
S_5	44.0	36.0	59	252	45.5	36.5	57	286	+13.4
S_6	45.0	36.0	55	357	48.0	37.5	53	415	+16.2
S_7	45.0	36.0	55	412	46.5	37.5	57	470	+14.0
S_8	45.5	36.0	57	390	48.0	38.5	55	476	+22.2

T_{db}=dry bulb temperature (°C); T_{wb}=wet bulb temperature (°C); Rh=relative humidity (%); E=sweat output ($g \cdot m^{-2} \cdot h^{-1}$); ΔE=increase of sweat output in percent of pre-trial test during the post-trial test. * S_3 had to interrupt the march after 1 day of exercise because of a knee injury.

Table 2. The 3 controlled hyperthermia tests in the 4 control subjects

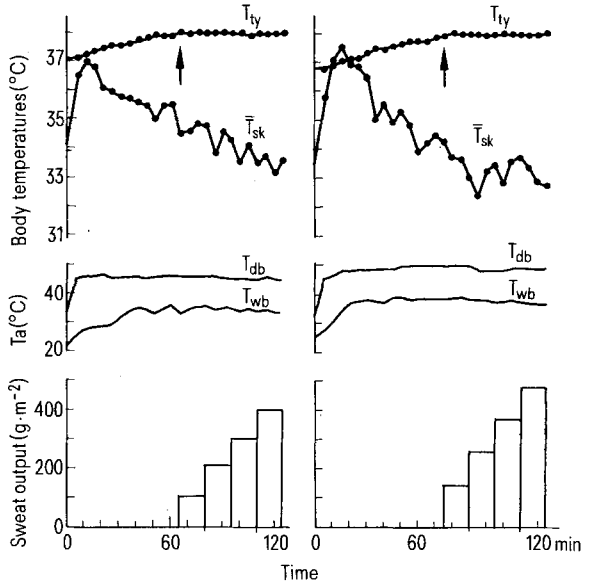
Subjects	1st test				2nd test				3rd test				Variations
	T_{db}	T_{wb}	Rh	E	T_{db}	T_{wb}	Rh	E	T_{db}	T_{wb}	Rh	E	HC_3/HC_1
C_1	46.5	35.0	47	325	46.5	35.0	47	326	49.5	36.0	42	326	+0.3
C_2	46.5	34.0	43	280	46.5	34.0	43	278	48.5	35.5	43	282	+0.7
C_3	46.0	36.0	51	363	46.0	36.0	51	363	49.0	37.5	48	382	+5.2
C_4	46.5	36.0	50	420	46.5	35.5	48	419	49.0	37.0	47	443	+5.4

T_{db}=dry bulb temperature (°C); T_{wb}=wet bulb temperature (°C); Rh=relative humidity (%); E=sweat output ($g \cdot m^{-2} \cdot h^{-1}$); HC_3/HC_1=percentage difference in sweat output during the 3rd controlled hyperthermia test (HC_3) as compared to the 1st controlled hyperthermia test (HC_1).

interval of 1 month). The 4 sedentary subjects of group 2 were tested thrice at monthly intervals. During the 1st and 2nd tests, the ambient conditions were similar to the pre-trial test of group 1. During the 3rd test, the ambient temperature was increased to reproduce the ambient conditions of the post-trial test of group 1 (see the results section).

Results. The ambient conditions and the individual heat responses of group 1 are given in table 1 and the figure is an example of the composite graphs summarizing the individual reactions throughout the tests. During the post-trial test, 7 subjects experienced an increase of sweat output (17.3% ± 1.5 SEM of the pre-trial values, $p < 0.001$). In 1 subject (S_3), the sweat output increased only 6.8%. Owing to a knee injury, S_3 interrupted the march after 1 day of exercise. It was also found to be impossible to reach the tympanic temperature target of 38 °C by using the same ambient conditions as in the pre-trial test. Therefore, because of the difficulty of reaching the 38 °C tympanic temperature target, the ambient temperature had to be set above the pre-trial test values (table 1). For group 2, ambient conditions and individual responses to the hyperthermia test are given in table 2. There was no difference between the 1st and 2nd tests. The sweat output was only slightly increased during the 3rd test (table 2) which was performed under warmer conditions and represented a replica of the post-trial test conditions of group 1.

Discussion. This study demonstrated that a 6-day march, with moderate energy expenditure, was capable of improving the sweating response of human subjects during a controlled hyperthermia test performed by passive heating in resting man. The increased sweat output observed after physical exercise (post-trial test) was possibly due to: a) an increased heat response from physical exercise; b) the increased ambient temperature necessary to reach the target tympanic temperature; c) a possible 'retention' maintained from the previous month's performance. However, the results obtained in the 4 sedentary subjects discard the latter possibilities. The absence of any difference between the 1st and 2nd heat exposure tests eliminates a possible 'retention' phenomenon. Furthermore, the sweat output was only slightly increased during the 3rd test in group 2. It can thus be concluded that the increased ambient temperature necessary to reach the target value after physical exercise was not responsible for the increased sweat output observed in the 7 exercising subjects. Therefore, the only explanation for the improved heat response was the benefi-



Body temperatures (tympanic: T_{ty}; mean skin: $\bar{T}_{sk}$), ambient temperatures (dry bulb: T_{db}; wet bulb: T_{wb}) and sweat output during the pre- (left graph) and post-trial (right graph) tests in subject 8. The arrow indicates when the T_{ty} target (38 °C) was reached. The cumulative sweat output is represented only during the 1-h hyperthermia, every 15 min. It can be seen that it was more difficult to reach the T_{ty} target during the post-trial test: the subject had to be exposed for a longer time at higher ambient temperatures. However, during the 1-h 38 °C-hyperthermia, the sweat output was consistently increased during the post-trial test.

cial effect of exercise on the sweating ability of these subjects. This was further confirmed by the results obtained in S_3.

This enhanced exercise-induced response to heat cannot be attributed to an improvement of $\dot{V}O_2max$ which did not alter during our field experiment in the 7 exercising subjects. Moreover, there was no relationship between $\dot{V}O_2max$ and the sweat output. Thus, the level of physical fitness does not seem to be related to the level of heat response. It appears that training, and not the level of fitness, is responsible for the improved heat reactions. This improvement could be due to modifications in the thermal balance provoked by moderate and prolonged exercise. This possibility is strengthened by the presence of a hyperthermia observed during the day ($+0.5 \pm 0.05$ °C; Kuehn, personal communication) and throughout the night of sleep during the exercise period[17]. The thermal balance change throughout the nycthemeral period may be responsible for an enhanced heat acclimatization. This hypothesis agrees with Belding's statement that an increase in core temperature is the essential stimulus for the processes of acclimatization to heat[18].

In conclusion, a moderate but prolonged physical training in a cool climate, without any variation in $\dot{V}O_2max$, was sufficient to improve heat acclimatization in young fit men, certainly through a slight but consistent increase in core temperature.

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Alfalfa seeds: Effects on cholesterol metabolism[1]

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Summary. Plasma cholesterol concentrations were reduced in 3 human volunteers during ingestion of diets containing alfalfa seeds (AS) for 3 weeks. No signs of toxicity were detected through serum determinations of multiple parameters. The ingestion of AS in rats decreased the concentration of plasma cholesterol, reduced intestinal absorption of exogenous and endogenous cholesterol, and increased fecal biliary excretion.

Alfalfa meal (sun-cured alfalfa hay) prevents hypercholesterolemia and atherosclerosis in cholesterol-fed rabbits[2]. A 50% concentration of alfalfa meal substituted isoenergetically in semipurified diets (SPDs) containing butter and cholesterol lowers cholesterolemia and plasma phospholipid levels, normalizes plasma lipoprotein distribution, and reduces the extent of aortic and coronary arteriosclerosis in cynomolgus macaques *(Macaca fascicularis)*[3]. The data on monkeys with an intake of saturated fat and cholesterol like that in the usual American diet suggests that alfalfa meal counteracts the hypercholesterolemic and atherogenic effects of dietary cholesterol[3]. In spite of its potential usefulness, it is unlikely that alfalfa meal may be ingested to a large extent by humans because of its taste and probable associated gastrointestinal disturbances. We report here studies on cholesterol metabolism performed with an alfalfa preparation that has been tolerated by humans. Preliminary findings were communicated previously[4].

Human experiments. Volunteers (3 women, ages 21–59, and 3 men, ages 43–59), informed of the experimental nature of the study, were initially given graded amounts of ground alfalfa seed (AS) to establish tolerance and seriousness of intent. The women declined to continue with the program because they a) lacked motivation, b) vomited because of concomitant therapy for hypertension, or c) feared gastrointestinal cancer might develop (1 woman in each category). The 3 men were subsequently given 160 g/day (AS 4,1) and 80 g/day (AS 8,3) in 2 trials of 3 weeks each. These preparations are described below. Alfalfa seeds consist of 88.3% dry matter, 4.4% ash, 8.1% crude fiber, 10.6% lipid, 32% N-free extract, and 33.2% protein ($N \times 6.25$); the caloric value for a monogastric omnivorous mammal is 340 cal/100 g[5].

Venous blood was withdrawn at weekly intervals for an initial baseline period, during administration of alfalfa seeds, and during the subsequent 3 weeks. The plasma cholesterol levels were determined by a modification of the $FeCl_3$ method[6]; high-density lipoprotein-cholesterol (HDL-cholesterol) was measured by the phosphotungstate precipitation method[7]. The following determinations were performed according to usual laboratory procedures on serum

Table 1. Plasma cholesterol values in 3 men preceding and following the ingestion of ground alfalfa seed (AS) (mean ± SE)

Preparation[a]	Intake (g/day)	Plasma cholesterol (mg/dl) Before AS	After AS
AS 4,1	160	248 ± 18	187 ± 14[b]
AS 8,3	80	253 ± 38	206 ± 35[c]

[a] See text. Student's paired t-test: [b] $p < 0.01$; [c] $p < 0.05$.