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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3 C.H. Wyndham, J. appl. Physiol. *4*, 383 (1951).
4 W.B. Strydom, C.H. Wyndham, C.G. Williams, J.F. Morrisson, C.A.G. Breddell, A.J.S. Benade and M. Van Radhen, J. appl. Physiol. *21*, 636 (1966).
5 C.H. Wyndham, A. Rev. Physiol. *35*, 193 (1973).
6 R. Hénane and J.L. Valatx, J. Physiol. *230*, 255 (1973).
7 R. Hénane and J. Bittel, J. appl. Physiol. *38*, 294 (1975).
8 R.W. Piwonka, S. Robinson, V.L. Gay and R.S. Manalis, J. appl. Physiol. *20*, 379 (1965).
9 R.W. Piwonka and S. Robinson, J. appl. Physiol. *22*, 9 (1967).
10 O.G. Edholm, Proc. R. Soc. Med. *62*, 1175 (1969).
11 C.V. Gisolfi, J. appl. Physiol. *35*, 349 (1973).
12 E. Baum, K. Bruck and H.P. Schennicke, J. appl. Physiol. *40*, 404 (1976).
13 R. Hénane, R. Flandrois and J.P. Charbonnier, J. appl. Physiol.; Respirat. envir. Exercise Physiol. *5*, 822 (1977).
14 H.B. Hale, Envir. Res. *2*, 423 (1969).
15 E.M. Glaser and R.J. Shephard, J. Physiol. *169*, 592 (1963).
16 W.S. Myles, J.P. Eclache and J. Beaury, Aviat. Space envir. Med. *50*, 921 (1979).
17 J. Bittel, A. Buguet, B. Roussel and R. Angus, in: Direction des Recherches et Etudes Techniques, Report No. 78/1198, p. 87.
18 H.S. Belding, in: Physiological Adaptations, p. 9. Ed. M.K. Youssef, J.M. Horvath and R.W. Bullard. Academic Press, New York 1972.

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] $p < 0.05$.

samples obtained before and after AS administration: triglycerides, glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, CO_2, uric acid, calcium, phosphorus, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase, lactic dehydrogenase, serum glutamic oxalacetic transaminase, and γ-glutamyl transpeptidase. Body weights were recorded weekly. The volunteers ingested AS suspended in fruit juice or a low-calorie soft drink, in divided doses before meals. No attempt was made to monitor the diet; however, each person was asked to adjust his usual diet to maintain a nearly constant body weight.

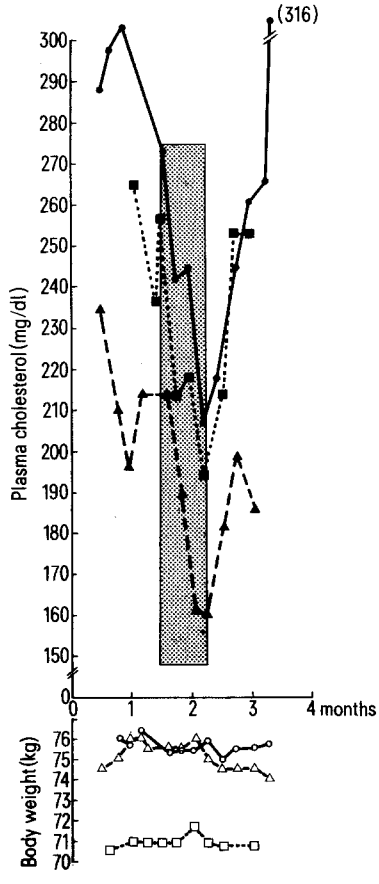
The alfalfa seeds were obtained from commercial sources that also raise alfalfa sprouts for the local market, a probable indication that no toxic additives were present. Initially, the seeds were ground with a Magic Mill food grinder (Magic Mill Center, Portland, OR); 76% was retained by a Tyler 45-gauge sieve (AS 4,1). Subsequently, the AS was more finely ground, and 12% was retained by the same sieve (AS 8,3).

Animal experiments. 4 groups of 6 male Sprague-Dawley rats each were meal fed from 08.00 to 10.00 h for 3 weeks: a) semipurified diet (SPD)[8]; b) SPD plus 5% AS 8,3; c) SPD plus 15% AS 8,3; or d) SPD plus 30% AS 8,3. On the day of the experiment, the animals were anesthetized with ether after feeding. A tail vein was cannulated with PE-10[9] for the injection of 5 µCi of [1,2-^{3}H]cholesterol, which was dissolved in alcohol and suspended in 1.0 ml of saline; 2 mg of cholesterol containing 2 µCi of [4-^{14}C] cholesterol dissolved in 0.5 ml of ethanol was given via stomach tube[8]. The rats were returned to their cages and given food and water ad libitum; feces were collected for 72 h, and then blood for cholesterol determinations was obtained from the tails of the rats, which were under ether anesthesia[6]. Fecal neutral steroids were measured as described elsewhere[8]. In addition the feces remaining after neutral steroid extraction were placed in a nickle crucible with 1 ml of 10 N KOH and saponified at 15 lb of pressure for 3 h. The residue was acidified with 3 ml of 10 N NCl, quantitatively transferred to a 50-ml screw-cap tube, and extracted 4 times with 15 ml of diethyl ether. The extracts were combined, dried under N_2, and dissolved in 1 ml of N-butanol:acetic acid:water (10:1:1, v/v). A 0.1-ml aliquot was transferred to a counting vial and 12 ml of 10% Beckman BioSolv (Beckman Instruments, Inc., Fullerton, CA) was added. Radioactivity was assayed[8], and calculations were performed with a computer program designed for dual isotope labeling[10].

Results and discussion. The volunteers ingested the suspended AS without difficulty. During the 1st week of ingestion, the men observed increased intestinal gas and bulkier feces that did not require discontinuation of AS. Individual results of the first trial with 160 g/day are shown in the figure. Table 1 shows the average values from tests performed with 160 and 80 g/day; the differences in plasma cholesterol levels before and after the ingestion of AS were significant with both intake levels. The levels of HDL-cholesterol were unchanged (45 ± 14 versus 43 ± 11 mg/dl). The body weights remained relatively constant throughout the observation period. Serum parameters enumerated above did not change consistently.

Results in rats (table 2) indicated that AS decreased the concentration of plasma cholesterol and its specific activity, and increased the excretion of ^{14}C- and ^{3}H-labeled neutral steroids and of bile acids.

These results may be considered preliminary since the number of subjects was small and they were studied for a short interval. Within these limitations, AS was accepted and well tolerated by half of the volunteers. Addition of the ground seeds to their habitual diets was associated with lowered plasma cholesterol levels without changes in HDL-cholesterol, whereas no humoral signs of toxicity were detected through serum determinations. The results ob-



Individual values of plasma cholesterol and body weight of 3 men. The shaded area corresponds to the period of ingestion of ground alfalfa seed (160 g/day).

Table 2. Parameters in 4 groups of rats (6 rats per group) fed different amounts of ground alfalfa seed (AS 8,3) (mean ± SE)

Regimen	Body weight (g)	Plasma cholesterol (mg/dl)	Plasma ^{3}H-cholesterol specific activity (dpm/mg/µCi)	^{14}C-neutral steroid excretion (% ID/µCi)[a]	^{3}H-neutral steroid excretion (% ID/µCi)[a]	^{3}H-bile acid excretion (% ID/µCi)[a]
Semipurified diet	361 ± 11	127 ± 14	4,637 ± 170	24.8 ± 0.8	1.2 ± 0.6	8.3 ± 0.8
Semipurified diet + 5% AS	347 ± 5	94 ± 6[b]	4,792 ± 200[b]	38.9 ± 3.0[c]	2.9 ± 0.3[d]	11.2 ± 0.8[b]
Semipurified diet + 15% AS	358 ± 6	84 ± 5[e]	3,898 ± 250[f]	39.1 ± 2.1[d]	2.4 ± 0.2[d]	16.7 ± 1.0[d]
Semipurified diet + 30% AS	356 ± 11	82 ± 5[e]	3,765 ± 80[d]	38.9 ± 2.2[d]	2.7 ± 0.2[d]	14.9 ± 1.4[e]

[a] ID = Injected dose; [b] not significant; [c] p (t-test) versus semipurified diet: < 0.01; [d] p (t-test) versus semipurified diet: < 0.001; [e] p (t-test) versus semipurified diet: < 0.02; [f] p (t-test) versus semipurified diet: < 0.05.

tained in rats suggest that the reduction in cholesterolemia may be secondary to a decrease in the intestinal absorption of exogenous and endogenous cholesterol and an increase in the excretion of bile acids. However, balance studies in humans are necessary to determine whether the mechanisms in rats and humans are comparable. The intestinal effects may be related to the inhibition of cholesterol absorption by alfalfa saponins[8,11] and to adsorption of bile acids to alfalfa fibre[12] with a consequent increase in their excretion. Moreover, additional hypocholesterolemic mechanisms associated with the minor dietary changes necessitated in our volunteers for conservation of constant body weight cannot be ruled out. The observations reported here suggest that the addition of alfalfa seeds to an habitual diet may prove useful in the treatment of hypercholesterolemic patients, but a larger number of volunteers should be followed for longer periods before this addition is recommended.

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2 F.B. Cookson, R. Altschul and S. Federoff, J. Atheroscler. Res. *7*, 69 (1967).
3 M.R. Malinow, P. McLaughlin, H.K. Naito, L.A. Lewis and W.P. McNulty, Atherosclerosis *30*, 27 (1978).
4 M.R. Malinow, 60th Annual Meeting, Pacific Division, American Association for Advancement of Science, University of Idaho, Moscow, 3–7 June 1979, p. 30.
5 Atlas of Nutritional Data on U.S. and Canadian Feeds p. 44. Natl Acad. Sci., Washington, D.C., 1972.
6 L.L. Rudel and M.D. Morris, J. Lipid Res. *14*, 364 (1973).
7 M. Burstein, H.R. Scholnick and R. Morfin, J. Lipid Res. *11*, 583 (1970).
8 M.R. Malinow, P. McLaughlin, L. Papworth, C. Stafford, G.O. Kohler, A.L. Livingston and P.R. Cheeke, Am. J. clin. Nutr. *30*, 2061 (1977).
9 J.L. Bollman and E. Van Hook, J. appl. Physiol. *24*, 722 (1968).
10 M.R. Malinow and P. McLaughlin, Atherosclerosis *15*, 153 (1972).
11 M.R. Malinow, P. McLaughlin, C. Stafford, A.L. Livingston, G.O. Kohler and P.R. Cheeke, Am. J. clin. Nutr. *32*, 1810 (1979).
12 D. Kritchevsky and J.A. Story, Am. J. clin. Nutr. *28*, 305 (1975).

Resilin in the cuticle of physogastric queen termites

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Summary. Resilin is present in the endocuticle of the flexible abdominal intersegmental cuticle of the physogastric queen of the termite, *Odontotermes obesus* (Rambur). Resilin seems to assist in the extension of abdomen of the queen termites during physogastry.

A characteristic feature of the physogastric queens of the tropical mound-building species of termites is the enormous growth (about 50-fold) of the abdomen after fertilization[3]. This is due to the increase in growth of visceral organs, and de novo development of abdominal tracheal glands and ovaries. During the extension of abdomen in the physogastric phase, the soft intersegmental membranes of the abdominal region grow and extend enormously[4]. Observations which we have recently made on the extended soft intersegmental membranes of the abdominal region of the physogastric queens of the mound-building termite, *Odontotermes obesus* (Rambur) indicate the presence of the structural elastic protein, resilin in the endocuticle which may assist in the elongation of abdomen during physogastric phase.

The soft intersegmental cuticle of the queen of *Odontotermes* comprises an outer epicuticle and inner endocuticle[4]. Histological preparations of the cuticle stained with toluidine blue/light green combination at pH 4–7 colours the endocuticle sapphire. In addition, the endocuticle swells considerably in phenol, formamide, formic acid, lithium thiocyanate and cupric ethylenediamine[5,6].

Chromatographic analysis for amino-acids of the intersegmental cuticle following the methods used by Bailey and Weis-Fogh[7] showed the presence of 2 fluorescent amino-acids (di-tyrosine and tri-tyrosine) at R_f 0.05 and 0.18 respectively, in addition to the usual amino acid constituents of the soft intersegmental cuticle of insects[4]. The fluorescence of these 2 amino-acids increased when the chromatogram was exposed to ammonia vapour, whereas vapour from hydrochloric acid quenched it almost completely.

Examination of the frozen sections of the soft intersegmental cuticle with fluorescence microscope (Carl Zeiss) showed that at neutral pH the outer half of the endocuticular region fluoresced blue with a maximum intensity at about 420 nm. In alkali media it fluoresced a brighter blue.

The foregoing observations, especially the presence of di- and tri-tyrosine as well as auto-fluorescence of the endocuticle provide characteristic evidence for the occurrence of resilin in the endocuticle of the soft intersegmental cuticle of the extended abdominal region of the physogastric queen of the termite, *Odontotermes*. It is suggestive that the resilin may impart elasticity to the cuticle necessary for the extension of abdomen during the physogastric phase.

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2 Previously called A. Sannasi.
3 W.A. Sands, Insectes soc. *12*, 117 (1965).
4 A. Sannasi, Thesis. Madras University, Madras, India 1969.
5 S.O. Anderson and T. Weis-Fogh, Adv. Insect Physiol. *2*, 1 (1964).
6 A. Sannasi, Experientia *26*, 154 (1970).
7 K. Bailey and T. Weis-Fogh, Biochem, biphys. Acta. *48*, 452 (1961).