inhibition is subserved by analogous neuronal pathways. An important difference between these segments consists in the occurrence of recurrent inhibition produced by contralateral antidromic volleys. In the lumbar cord motoneurones are only inhibited by ipsilateral conditioning[2,9]. However, in the sacral cord both somatic and parasympathetic neurones are affected by contralateral volleys. Our preliminary experiments on the effect of 5-hydroxytryptophan on somatic recurrent inhibition in the S 3 segment suggest that it may be bilateral[8]. De Groat and Ryall[10] found that antidromic stimulation of the ventral roots containing parasympathetic preganglionic fibres depressed spontaneous contractions of the bladder. Although this depression was observed in preparations in which only crossed effects could be studied, it is very probable that autonomic recurrent inhibition is bilateral.

It remains to be elucidated whether the shorter time course of recurrent inhibition evoked in the Ca 1 segment by contralateral conditioning depends on specific properties of the pathway transmitting antidromic inhibitory action to the opposite side of the cord. Since the caudal segments together with the lower sacral segments innervate the midline structures of the body, bilateral recurrent inhibition provides the best coordination of the motor function of symmetrical halves of the cord.

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Cellularity and composition of epididymal adipose tissue from cold-acclimatized rats[1]

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Summary. Cold acclimatization induces morphological and compositional modifications of rat epididymal adipose tissue: a decrease in fat cell size, an increase of fat cell number per g of tissue, but no significant increase in total fat cell number in the tissue; finally, an increase in protein content and a decrease in triglyceride content.

The epididymal adipose tissue from cold-acclimatized rats (CA) shows an increased capacity of synthesizing fatty acids from acetate[2] and an increased lipolytic potential in response to norepinephrine[3]. In agreement with these observations, in this laboratory it was found[4] that cold acclimatization induced a twofold increase in the turnover rate of fatty acids in the triglycerides of the rat epididymal adipose tissue. This result led us to investigate the effects of 4 weeks of cold-exposure upon the cellularity and the chemical composition of rat epididymal adipose tissue in order to determine if morphological and compositional alterations were associated with the metabolic ones previously described.

Materials and methods. Experiments were performed on Long Evans male rats. The animals were acclimatized to different thermic conditions when 7 week old: the control group was maintained at 28 °C (thermal neutrality) for 3 weeks; the cold-acclimatized group (CA) was exposed to a constant temperature of 5 °C for 4 weeks. This schedule resulted in 2 groups of mean b.wt between 280 and 305 g. The animals were maintained on a daily 12-h dark-light cycle and fed on a standard laboratory diet with water ad libitum until sacrifice.

Isolated fat cells were prepared according to the procedure of Rodbell et al.[5], with minor modifications: the absence of glucose, a lowering of the collagenase concentration (5 mg/g adipose tissue), a decrease in the dissociation period (45 min) and 2 cell washing procedures instead of 3.

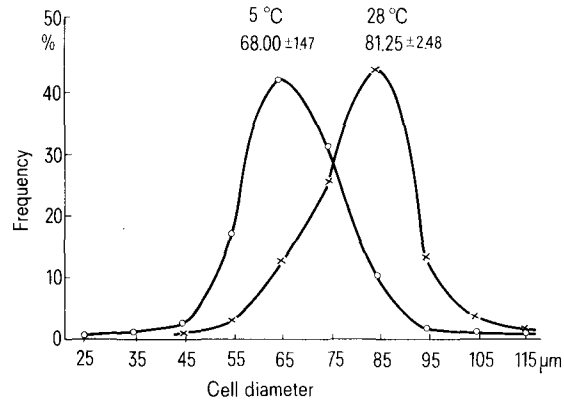
Fat cell size was determined according to the photomicrographic method described by Lavau et al.[6]. The fat cell number was determined using the method of Di Girolamo et al.[7]. Fat cell yields (approx. 80%) did not differ significantly between the 2 groups.

Total lipids were extracted from tissue samples by the procedure of Folch et al.[8]. Aliquots of lipid extracts were analysed for phospholipid phosphorus using Bartlett's method[9]. After hydrolysis and selective precipitation by digitonin, as described by Sperry and Webb[10], additional aliquots were analyzed for cholesterol content by ZAK's technique[11].

Triglyceride (TG) content (percent wet weight of tissue) was calculated as follows: TG content = total lipid content (percent wet weight of tissue) − [phospholipid content (percent wet weight of tissue) + cholesterol content (percent wet weight of tissue)].

Protein content was determined by the method of Lowry et al.[12] after precipitation by 100% perchloric acid.

Results and discussion. Effects of cold acclimatization on the diameter distribution pattern of epididymal fat cells. A diameter distribution pattern of epididymal fat cells from CA rats and controls is shown in the figure. Cold acclimat-



Epididymal fat cell diameter distribution patterns of CA and control rats. The values shown at $\bar{X} \pm$ SEM represent the mean fat cell diameter for 4 rats in each group.

Effects of cold acclimatization on epididymal fat pads weight and composition, on fat cell size and number

	Controls (28 °C)	Cold-acclimatized (5 °C)	Signification
Body weight (g)	304 ±4 (4)	280 ±3 (4)	$p<0.01$
Epididymal fat weight (g)	2.41 ±0.11 (4)	1.95 ±0.08 (4)	$p<0.01$
Epididymal weight/body weight (%)	0.79 ±0.03 (4)	0.70 ±0.01 (4)	$p<0.05$
Mean fat cell diameter (μm)	81.25 ±2.48 (4)	68.00 ±1.47 (4)	$p<0.001$
Mean fat cell triglyceride content (μg)	0.273±0.013 (4)	0.161±0.010 (4)	$p<0.001$
Fat cell number/g adipose tissue × 10^6	3.17 ±0.17 (4)	4.47 ±0.32 (4)	$p<0.01$
Total fat cell number × 10^6	7.59 ±0.31 (4)	8.78 ±0.92 (4)	N.S.
Protein content (% wet weight)	0.59 ±0.04 (6)	0.97 ±0.06 (3)	$p<0.01$
Phospholipid content (% wet weight)	0.14 ±0.01 (5)	0.19 ±0.02 (6)	N.S.
Cholesterol content (% wet weight)	0.04 ±0.01 (4)	0.05 ±0.01 (6)	N.S.
Triglyceride content (% wet weight)	85.6 ±1.2 (5)	79.3 ±1.0 (6)	$p<0.01$

Values are the mean ± SEM. Numbers in parentheses indicate the number of determinations.

ization displaced the fat cell diameter distribution pattern towards smaller diameter values; it induced an important decrease in the mean fat cell diameter as compared to controls. As previous results from this laboratory (in press) suggested that cold acclimatization might increase the osmotic fragility of fat cells by decreasing their plasma membrane cholesterol content, it was decided to test whether washing procedures following collagenase treatment affect differently the cell diameter distribution pattern from one group to the other. No effect of the washing procedures was observed on this parameter in the two experimental groups; thus, it was concluded that the comparison of their mean fat cell diameter after washing procedures was reliable.

Effects of cold acclimatization on epididymal fat pad weight, fat cell size and number. The table indicates the differences in epididymal fat pad weight and cellular characteristics between the CA group and the control one. Cold acclimatization decreased epididymal fat pad weight by 19% ($p < 0.01$). Consequently, the epididymal adipose tissue contribution to total b.wt was significantly lower ($p < 0.05$) in CA rats. Cold acclimatization decreased mean fat cell diameter by 16% ($p < 0.001$), resulting in a 41% decrease in the mean fat cell triglyceride content ($p < 0.001$). This result confirms that reported by Therriault et al.[13], who observed a decrease of 47%. Nevertheless, the absolute values for the mean TG fat cell content reported by the last authors are about 4 times lower than those observed here, although the mean b.wt of the animals studied was approximately the same: 320 g. This discrepancy probably results from the difference in methods used for fat cell size determination. In our study, the fat cell diameter was directly determined by means of a reliable photomicrographic method recently described by Lavau et al.[6]. In that of Therriault et al.[13], fat cell size determination was based on the calculation of total fat cell number, the value of which seems greatly overestimated when compared with those reported by others for animals of the same age[14–16].

Fat cell number/g of epididymal adipose tissue was increased by 41% ($p < 0.01$) in CA rats, but the total fat cell number was not significantly increased by cold acclimatization. The lack of a significant cold acclimatization effect upon fat cell number may be related to the stage of development of the rats when exposed to cold (7 weeks). The rats used in this study had probably passed the primary hyperplastic state of fat depot development (6 weeks)[14]. Cold acclimatization took place during the stage of simultaneous hyperplasia and hypertrophy (6–15 weeks)[14]; thus, it could have exerted a lesser effect on total fat cell number than in the study of Therriault et al.[17], where rats were acclimatized to 5 °C when 3–4 weeks old.

Effects of cold acclimatization on epididymal adipose tissue composition (table). Cold acclimatization increased protein content by 64%; this result supports that of Bertin et al.[18]. The increase in protein content may be related to the considerable increase of fat cell number/g adipose tissue. The increase in phospholipid (35%) and cholesterol (11%) contents did not reach statistical significance. These results show that cold acclimatization does not increase the main structural fat cell components in the same proportion. They coincide with the observations provided by a study of the chemical composition of cold-acclimatized rat plasma membrane fraction (in press): a significant decrease of the phospholipid/protein and cholesterol/protein ratios was found in this fraction, which led us to postulate that cold acclimatization induced both increased membrane activity and permeability.

Cold acclimatization decreased TG content by 7% ($p < 0.01$). This decrease may be related to a decreased capacity of TG storage in cold-acclimatized rat adipose tissue, due to a greater lipid utilization, as shown by an increased turnover rate of their esterifying fatty acids[4].

Cold acclimatization of the adult rat induces changes in the chemical composition and the cellular characteristics of epididymal adipose tissue but does not significantly increase total fat cell number. It is concluded that significant morphological and compositional modifications are associated with the increased metabolic activity of this tissue.

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