"External" tissue refers to tissue stripped from the exterior of a specimen with Teflon-coated instruments. "Internal" tissue refers to tissue sampled from the interior of a specimen, which was less susceptible to contamination during autopsy. The nickel concentrations in the external lung tissues exceed the concentrations in the internal lung tissues in three of the five lung pairs studied. There is a suggestion that contamination might have occurred in some cases, but the overall evidence is inconclusive. It is possible that, if the tissues were contaminated, this test might not reveal the contamination due to migration of the nickel during room temperature dissection. In the five kidney pairs studied, there is no evidence that the specimens were contaminated.

The results of the correlation study between the nickel levels in lung and the weights of the lung specimens taken at autopsy and sent to the Toxicology Branch of the CDC are shown in Fig. 1b. There is a significant correlation between the nickel levels and the sample weights in the Legionnaire cases, the high levels being associated with small specimen weights. No significant correlation is evident in the controls.

A model based on surface contamination of cubically or spherically shaped lung specimens would permit us to predict a slope of -0.33 in Fig. 1b. A model based on a constant amount of contamination per specimen would permit us to predict a slope of -1.0. For uncontaminated specimens, a slope of zero is expected. We found a slope of -0.46 ± 0.09 for the Legionnaire lung cases and a slope of 0.09 ± 0.30 for the controls (Fig. 1b). A similar log-log plot for the Legionnaire kidney tissues gave a slope of -0.17 ± 0.34 and a correlation coefficient of $r = -.18$ with $P = .64$ (*8*).

These results suggest contamination of the Legionnaire lung specimens but not of the kidney specimens. We examined autopsy records for possible differences in the handling of the lung and kidney specimens and found no recorded systematic differences in handling that could explain why the correlation was observed in the lung but not in the kidney specimens. However, since metal knives and scissors were used during the autopsies, contamination could have occurred preferentially, for example, if more incisions were made in examining consolidation in the lungs.

In the comparison of the measured nickel concentrations in the Legionnaire cases compared to the Eucharistic Congress cases and the controls, it should be noted that in seven of the Legionnaire cases reported here (L2, L3, L5, L6, L7, L8, and L9) the date of tissue samplings occurred on 2 August 1976. These samplings were done during the period when the extent of the epidemic began to be recognized, not after it was known that an epidemic was under way, as was the case for the Eucharistic Congress deaths and the controls. Accordingly, greater care may have been taken in handling the tissues from the Eucharistic Congress cases and the controls.

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The Cell Membrane Sodium Pump as a Mechanism for Increasing Thermogenesis During Cold Acclimation in Rats

Abstract. *Increased sodium pump activity is a major component of enhanced tissue thermogenesis in skeletal muscle, liver, and kidney of cold-acclimated rats. The sodium pump may play a major role in the thermoregulatory heat production during cold adaptation in mammals.*

When mammals are placed in a cold environment they lose a greater amount of heat than they do in a warm environment because of the increased difference between body temperature and ambient temperature. To maintain constant body temperature during exposure to cold they compensate for heat loss; that is, they acclimate to the colder environment by decreasing heat loss (through vascular and insulation changes) and by increasing metabolic heat production. During continuous exposure of the rat to moderate cold, shivering is the initial calorigenic response and is gradually replaced by nonshivering thermogenesis (NST). Nonshivering thermogenesis, which is the term applied to the increase in metabolic rate as a result of exposure to cold, does not involve muscle contraction (*1*). The proportion of shivering thermogenesis, NST, and heat loss changes utilized to maintain homeothermy varies with the species of mammal. The mechanisms of NST, their physiological controls, and the sites of NST are controversial problems (*1, 2*).

Recent studies of the mechanisms of cellular thermogenesis provide important data applicable to the NST of cold adaptation. Ismail-Beigi and Edelman (*3*) demonstrated the significance of the cell membrane sodium pump (Na^+ and K^+ transport system involving Na^+- and K^+-dependent adenosinetriphosphatase) (E. C. 3.6.1.3) as a major mechanism of thyroid hormone calorigenesis. The utilization of adenosine triphosphate (ATP) by the Na^+ pump increases oxidative metabolism and consequent liberation of metabolic heat. These authors suggested the possible involvement of the Na^+ pump as a mechanism for heat production during cold adaptation in mammals. Some data in the literature indicate that Na^+ pump activity is increased in the liver (*4*) and diaphragm (*5*) of cold-acclimated rats, and in the liver and skeletal muscle of mice (*6*), and thus support Ismail-Beigi and Edelman's suggestion.

The purpose of the present investigation was to determine the significance of heat production associated with active Na^+ transport from various tissues of cold-acclimating rats and to correlate this with the phenomenon of NST.

Male Wistar rats (30 to 50 days old) were paired with littermates on the basis of their initial weight and housed in individual cages. One of each pair was exposed to cold (6° ± 1°C), while the control was kept at room temperature

(26° ± 2°C). All animals were maintained on Purina Lab Chow and were given free access to water. After the exposure period the animals were killed and tissues were transferred to iced, oxygenated, modified sodium-Ringer solution (*7*). Animals were killed at approximately the same time each day of experimentation (9 a.m. ± 1 hour) to avoid diurnal variations.

Oxygen consumption was the indirect calorimetric parameter measured to estimate heat production. Tissue slices were prepared with a Stadie-Riggs hand microtome, and were transferred to a Gilson differential respirometer where oxygen consumption, QO_2, was measured at 15- to 30-minute intervals at 37°C for 1 hour by means of standard manometric procedures (*8*). Respiration that was independent of Na^+, QO_2', was measured in parallel incubation of tissue slices in Na^+-free solution prepared by isosmolar substitution of choline chloride or sucrose for the NaCl. In the experiments with pectoral muscle, liver, and kidney, the Na^+-free Ringer solution was supplemented with KCl to 20 m*M* K^+ (*9, 10*). Respiration that was dependent on Na^+, $QO_2(t)$ (respiration associated with active Na^+ transport), was calculated as the difference between total respiration and the Na^+-independent respiration (*3*). The difference in oxygen consumption between tissue slices with and without Na^+ in the incubation medium has been demonstrated to be equivalent to the difference when ouabain (a specific inhibitor of Na^+- and K^+-dependent adenosinetriphosphatase) is added or potassium omitted from the solution and may be taken as an indication of the energy utilized by transport Na^+- and K^+-dependent adenosinetriphosphatase (*3, 10, 11*). Tissue dry weights were determined after they were heated for 24 hours at 90°C.

Skeletal muscle is estimated to contribute about 50 percent of the NST of cold-acclimated rats (*12*). Because metabolic rates and NST may be different for different muscles, experiments with pectoral and diaphragm muscles were performed. The rat hemidiaphragm preparation is an intact tissue and its use eliminates the effects of tissue damage on cellular metabolism. Exposure of rats to cold temperatures for 7 to 50 days (period of metabolic acclimation; see Figs. 1 and 2) increased the respiration of tissue slices from diaphragm and pectoral muscle 9.2 and 27.2 percent, respectively, compared to tissue slices from control rats (Table 1). More important is the finding that the increased diaphragm and pectoral muscle tissue respiration of cold-acclimating rats is largely due to increased Na^+-dependent respiration, amounting to 83.3 and 70.6 percent of the increased tissue thermogenesis. The Na^+-independent respiration remained unchanged after cold acclimation in all tissues studied (Table 1). The responses of the two muscles with respect to the time of exposure to cold (Fig. 1) were similar, with the pectoral showing a greater magnitude of increase. The parameters of muscle tissue response observed in Fig. 1 are similar to those reported by Davis *et al.* (*13*). The stimulation of muscle tissue respiration and the continued thermogenesis during cold acclimation are thus largely due to increased Na^+-dependent respiration (that is, increased ATP utilization by the Na^+ pump increases oxidative metabolism with subsequent increase of metabolic heat production).

Tissue respiration of liver and kidney was also significantly increased for the total number of animals exposed to cold for periods greater than 7 days (Table 1). Some investigators have suggested that the liver is not a major contributor to NST in the rat (*14*). However, in recent studies it has been estimated that the liver can be responsible for as much as 25 to 35 percent of the increased oxygen uptake of cold-acclimated rats (*15*). In these studies it was determined that blood flow is increased to the liver, kidney, and other visceral organs during cold acclimation, and that these tissues can account for a greater proportion of total NST in the cold-acclimated rats than in controls. This also suggests that in our study, the increase in metabolism observed for liver and kidney tissue in vitro may be less than the actual calorigenic potential of these tissues in vivo during cold acclimation. What is important is that 95.6 percent of the increased liver tissue thermogenesis and 100 percent of the increased kidney response are associated with increased Na^+ transport. Whether the small (8.5 percent), yet statistically significant, increased tissue thermogenesis of the kidney (Table 1) is significant to the animal remains uncertain. However, the high metabolic rate of the kidney suggests its possible thermogenic importance. The sudden and significant ($P < .05$) increase in the kidney response on day 21 is diffi-

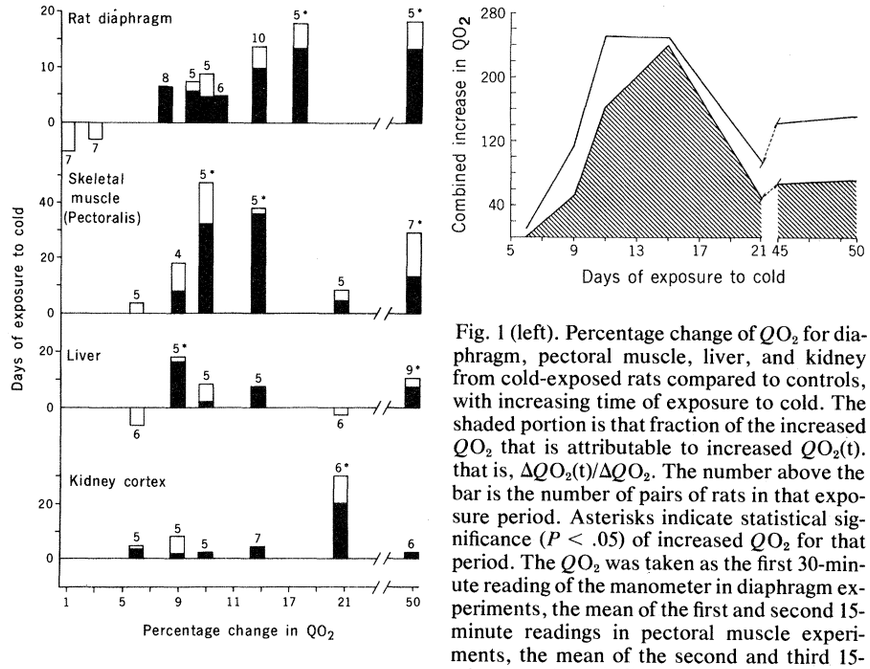

Fig. 1 (left). Percentage change of QO_2 for diaphragm, pectoral muscle, liver, and kidney from cold-exposed rats compared to controls, with increasing time of exposure to cold. The shaded portion is that fraction of the increased QO_2 that is attributable to increased $QO_2(t)$. that is, $\Delta QO_2(t)/\Delta QO_2$. The number above the bar is the number of pairs of rats in that exposure period. Asterisks indicate statistical significance ($P < .05$) of increased QO_2 for that period. The QO_2 was taken as the first 30-minute reading of the manometer in diaphragm experiments, the mean of the first and second 15-minute readings in pectoral muscle experiments, the mean of the second and third 15-minute readings in liver experiments, and the mean of the four 15-minute readings in kidney experiments. These time periods were used because they showed greater stability of the manometer readings for the various tissues. In preliminary studies and reported by Asano *et al.* (*10*) the tissue metabolism of the rat hemidiaphragm preparation varied inversely with tissue thickness when rats weighed more than 150 g. Therefore, only animals weighing less than 150 g at the time they were killed were used in diaphragm experiments. The values for diaphragm respiration on day 50 are for sliced preparations. Fig. 2 (right). The combined increase of total oxygen consumption (microliters per hour × body weight$^{.75}$) due to cold exposure calculated for the entire skeletal muscle mass, liver, and kidneys of the animals from Fig. 2, with increasing days of cold exposure. The shaded portion is that fraction of the increased oxygen consumption that is attributed to increased $QO_2(t)$. These data were determined by: (i) calculating the change in QO_2 due to cold exposure for each tissue at each period on the basis of wet weight of tissue (*19*); (ii) determining the percentage weight contribution of each tissue mass to the mean body weight of the group [changes in fractional weights of tissue masses occur with cold exposure; therefore, the fractional weights of the tissues were extrapolated from values given in (*20*)]; and (iii) calculating the metabolic increase for each of the three tissue masses and summing these values for each period of exposure.

Table 1. Mean (± standard error) tissue respiration (QO_2), mean Na^+-independent respiration (QO_2'), and mean Na^+-dependent respiration [$QO_2(t)$], in microliters per milligram of dry weight per hour, for diaphragm, pectoral muscle, liver, and kidney of rats exposed to cold (6°C for 7 to 50 days) and their littermate controls (26°C). Comparison was made between cold-exposed and control rats to determine the response due to cold. Significance (*) was determined by means of the Student's t-test ($P < .05$) for paired data. The ratio $\Delta QO_2(t)/\Delta QO_2$ is that fraction of the increased tissue respiration that is attributable to increased Na^+-dependent respiration.

Conditions	Mean respiration			Ratio of $\Delta QO_2(t)$ to ΔQO_2 (%)
	QO_2	QO_2'	$QO_2(t)$	
		Diaphragm (44 pairs)		
Control	1.97 ± .446	1.14 ± .319	0.83 ± .342	
Cold	*2.15 ± .453	1.17 ± .319	*0.98 ± .458	83.3
Increase (%)	9.2	2.6	18.1	
		Pectoral muscle (26 pairs)		
Control	1.25 ± .375	0.82 ± .339	0.43 ± .331	
Cold	*1.59 ± .495	0.92 ± .319	*0.67 ± .381	70.6
Increase (%)	27.2	12.2	55.8	
		Liver (30 pairs)		
Control	2.99 ± .752	2.58 ± .762	0.41 ± .531	
Cold	*3.22 ± .735	2.59 ± .665	0.63 ± .731	95.6
Increase (%)	7.7	0.4	53.7	
		Kidney (34 pairs)		
Control	5.74 ± 1.63	5.05 ± 1.42	0.69 ± 1.27	
Cold	*6.23 ± 2.20	5.05 ± 1.53	*1.18 ± 1.39	100
Increase (%)	8.5	0.0	71.0	

cult to interpret (Fig. 1). The different oscillating patterns of increased thermogenesis from the various tissues reported here could explain some of the controversy concerning which tissues are involved in NST, and to what extent they participate.

An estimate of the combined respiratory response of the entire muscle mass, liver, and kidneys was made in order to obtain some representation of the animal response to cold. This was necessary in view of the different patterns of tissue activation (Fig. 1), metabolic rates (magnitude of heat production), and fractional weight contribution of the tissues to total body weight of the rat. The calculated results (Fig. 2) of composite tissue thermogenesis of the skeletal muscle mass (based on pectoral muscle data), liver, and kidneys, are used here as a guide to the pattern of total NST of these tissues and do not necessarily represent the magnitude of the whole animal response (*16*).

In the rat, NST begins (as determined by whole animal metabolism in the absence of shivering) during the first week of cold exposure, increases during the second week, and reaches the higher levels by the third week (*17*). Figure 2 shows that this pattern corresponds to the tissue response of cold-acclimating animals. The magnitude of increased respiration of the tissues in Fig. 1 is consistent with the more recent reports of an increased basal metabolic rate of approximately 20 percent for cold-acclimated rats (*18*). Figures 1 and 2 indicate that the increase and continuation of tissue respiration during cold acclimation is correlated with Na^+ dependency. The increased metabolic rate of cold-acclimating rats may therefore be mediated through increased Na^+ pump activity of the tissues, and additional regulatory NST, either directly or indirectly, may involve this increased thermogenic property of the cell. The increased Na^+ pump activity could be a result of: (i) an increase in Na^+- and K^+-dependent adenosinetriphosphatase per unit of tissue mass (synthesis of new pump sites, unmasking of pump sites, or an increase in the ratio of cell surface to volume); (ii) alteration of the ion fluxes or ion concentration in the cell (membrane permeability or cotransport changes); or (iii) direct stimulation of the Na^+- and K^+-dependent adenosinetriphosphatase enzyme system. One or more of these factors are probably involved, either simultaneously or sequentially, in the acclimation process, and they may be mediated through hormone action.

Because most of the increased tissue thermogenesis of cold-acclimating rats is Na^+-dependent, we suggest that active Na^+ transport, by way of the cell membrane Na^+ pump, is a major mechanism for increasing the thermoregulatory NST of the rat and possibly other mammals.

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