Table 1. The concentration of NE, activity of DBH, and the pulse rate in 14 Lesch-Nyhan patients and 14 volunteer subjects. Results are expressed as means ± standard error. The basal concentration of NE was determined in supine subjects about 15 minutes after venipuncture. The increment in NE was determined by subtracting the basal NE from the concentration determined after the subjects were allowed to sit upright (two patients were tilted to 60 degrees).

Subject	NE (pg/ml)		Pulse rate		DBH (units of activity)*
	Basal	Increment	Basal	Upright	
Lesch-Nyhan	321 ± 57	39 ± 26†	106 ± 4‡	117 ± 3‡	344 ± 72†
Volunteers	248 ± 28	165 ± 40	74 ± 3	80 ± 3	668 ± 109

*One unit equals 1 nmole of phenylethylamine converted to phenylethanolamine per milliliter of plasma per hour. †$P < .02$, Student's t-test. ‡$P < .001$.

As expected, the Lesch-Nyhan patients were more agitated than the volunteer group, and restraints were used with several patients. It is likely that these patients did not understand the procedure but were afraid that they were to undergo a painful experience. The basal pulse rate in the patients was significantly higher than controls (Table 1). In normal subjects, pulse rate and plasma levels of NE decline significantly and proportionately between immediate and basal samplings (*7*). In the present experiments, the pulse rate of the patients declined significantly ($P < .05$; paired t-test) by 10 ± 4 beats per minute to 116 ± 6 by about 15 minutes after venipuncture, but the patients did not reach basal state as observed subjectively or objectively by pulse rate at the time the basal samples were obtained. The concentration of NE in the plasma obtained immediately after venipuncture was 331 ± 67 pg/ml, and there was a minimal decrease in this concentration by the time the second (basal) sample was obtained. This decrement was not significant; also, the NE concentrations in the plasma obtained immediately or 15 minutes after venipuncture were not significantly higher than the basal levels of NE from the volunteers, despite the higher anxiety levels of the patients (Table 1). After the postural change the patients maintained their elevated state of anxiety and their pulse rates remained higher than controls ($P < .001$; Table 1). Although the concentrations of NE in the plasma obtained from patients and volunteers in the upright position were similar (360 ± 84 and 413 ± 46, respectively), the increment in plasma NE in the Lesch-Nyhan patients was significantly blunted (Table 1). In a normal population the NE concentrations increase by about 65 percent above basal values after the individuals have been sitting or have been tilted to 60 degrees for 10 minutes, regardless of the magnitude of the basal plasma content of NE (*7*, *9*).

In contrast to previous results (*4*), the present study showed that the DBH activity in 14 Lesch-Nyhan patients was significantly lower than that in either the 14 volunteers ($P < .02$; Table 1) or the large adult control group (760 ± 26 units; $P < .001$). To determine whether Lesch-Nyhan plasma contains an inhibitor of DBH activity, the plasma from nine patients was individually mixed with an equal volume of plasma containing high DBH activity and allowed to stand at room temperature for time periods of 5 and 60 minutes before being assayed for DBH activity. The DBH activities from the mixed plasma samples were found to be additive at both time periods, suggesting that the low activity in Lesch-Nyhan plasma is not secondary to endogenous inhibitors.

Since Lesch-Nyhan patients are not able to stand up unassisted, sitting in their wheelchair is generally the most upright posture they assume with respect to the force of gravity on the circulatory system. A major function of the peripheral noradrenergic nervous system is the maintenance of blood pressure upon standing by increasing peripheral vascular resistance. The activity of DBH has been demonstrated to be lower during sleep (*12*) compared to the waking hours when posture is often changed and individuals are upright. The present data, showing (i) the absence of an elevation in plasma NE concentrations in patients with Lesch-Nyhan syndrome immediately after venipuncture, (ii) a diminished increment in NE upon postural change, and (iii) low plasma DBH activity, support the recent report (*4*) of a diminished autonomic responsivity to cold in Lesch-Nyhan syndrome.

The explanation for these data in Lesch-Nyhan patients may be the relative disuse from infancy of the sympathetic nervous system because of the lack of demand for noradrenergic responsiveness to standing posture, this resulting in a diminished need for synthesis of NE and its synthetic enzymes. Basal noradrenergic tone is not reflected by plasma DBH activity in Lesch-Nyhan syndrome because basal concentrations of NE are normal. That these patients raised their heart rates under conditions of stress supports the view (*13*) that the cholinergic nervous system has a dominant role in control of heart rate during stress.

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Legionnaires' Disease: Nickel Levels

Abstract. *Nickel concentrations in lung tissues of nine deceased Legionnaire victims average nine times that of controls. There is a significant correlation between the nickel levels in the lungs of the Legionnaire victims and the weights of the lung specimens. No similar correlation is evident in the study of the victims' kidney tissues or tissues from the controls.*

Since the outbreak of Legionnaires' disease during July and August 1976, medical sleuths have indicated that a toxin may have been the responsible agent. Suggested toxins included nickel carbonyl, phosgene, paraquat, and others (*1*). The phosgene and paraquat theories are based on inferences from the

symptomatic and pathologic findings in the various cases. The nickel carbonyl hypothesis appears amenable to a more direct test by measurement of the nickel concentrations in the victims' tissues.

We report here the nickel concentrations measured in lung and kidney tissues of Legionnaires' disease cases and controls. We found that the nickel in the Legionnaire lung tissues averaged nine times that in controls (117 compared to 13 μg/100 g, dry weight). We found a significant correlation between the nickel in the lungs of the victims and the weights of the lung specimens taken at autopsy and sent to the Toxicology Branch of the Center for Disease Control (CDC). No correlation was evident in the Legionnaire kidney tissues or in tissues from the controls. As a test for contamination we measured the nickel in samples taken from the interior and exterior of the larger specimens; the results were inconclusive.

We used the method of proton-induced x-ray emission (*2*) to measure the nickel in the lung and kidney tissues. Samples (0.1 to 0.5 g) of tissue were weighed in sterile, capped plastic test tubes. Concentrated nitric acid (Baker "Ultrex") was added, and the samples were heated to promote digestion. Portions (10 μl) of the solutions were evaporated to dryness and analyzed directly in a 2-Mev proton beam. As was described (*3*), proton-induced characteristic x-rays, including those of nickel at 7.472 kev, were detected in a Si(Li) detector, and the nickel concentrations were determined. We performed the quantitative calibration and cross-check measurements by analyzing samples to which known concentrations of nickel had been added.

Table 1. Nickel levels in lung and kidney tissues of the Legionnaire cases, controls, the "Broad Street Pneumonia" (BSP) and Eucharistic Congress cases.

Patient	Nickel in tissue (μg/100 g, dry weight)	
	Lung	Kidney
Legionnaire Cases		
L1	65	3
L2	24	
L3	52	12
L4	86	26
L5	120	17
L6	82	
L7	399	
L8	87	
L9	139	
L10		10
L11		20
Controls		
C1	12	5
C2	10	13
C3	31	12
C4	6	9
C5	5	15
C6	5	21
C7	29	
C8	9	
C9	12	
Other cases		
N1 (BSP)	12	9
N2 (Congress)	21	5
N3 (Congress)	17	11

The identities of the patients studied were released to us by the CDC (*4*) after our test results were submitted to the CDC in conformity with coded protocol.

The nickel concentrations in the lungs of the Legionnaire cases L1 to L9 and those for the controls C1 to C9 are shown in Table 1. The average concentration for the Legionnaire cases is nine times that of the controls, that is, 117 compared to 13 μg/100 g (dry weight), respectively. These means are significant at $P = .02$. These nickel concentrations in lungs for the Legionnaire cases are in agreement with those reported (*5*) for cases L2, L3, L5, L6, and N1 and are comparable with the 66 (*6*) and 115 (*5*) μg/100 g, dry weight, found in confirmed cases of acute nickel carbonyl poisoning [compared to 4.3 to 14.6 μg/100 g in control patients (*5*)].

Additional data have also been obtained in three other cases (Table 1): patient N1, who met the clinical criteria (*7*) defining a disease case and had been within a block of the Bellevue Stratford Hotel but had not entered it since 1 July 1976—this case has been categorized by the CDC as a "Broad Street Pneumonia (BSP)" case; patients N2 and N3, who attended the Catholic Eucharistic Congress during 1 to 8 August 1976—the pathology reports on these two cases indicate that they should be classified separately from the American Legion Convention cases. The nickel concentrations in the lungs of these three cases are substantially lower than those in the Legionnaire cases' concentrations.

The nickel concentrations measured correspond to only 1 to 40 μg of nickel in the autopsy specimens. Such small amounts could have been introduced inadvertently, and hence the question of contamination of the tissues must be addressed.

Figure 1a shows the results of an "external-internal" contamination test.

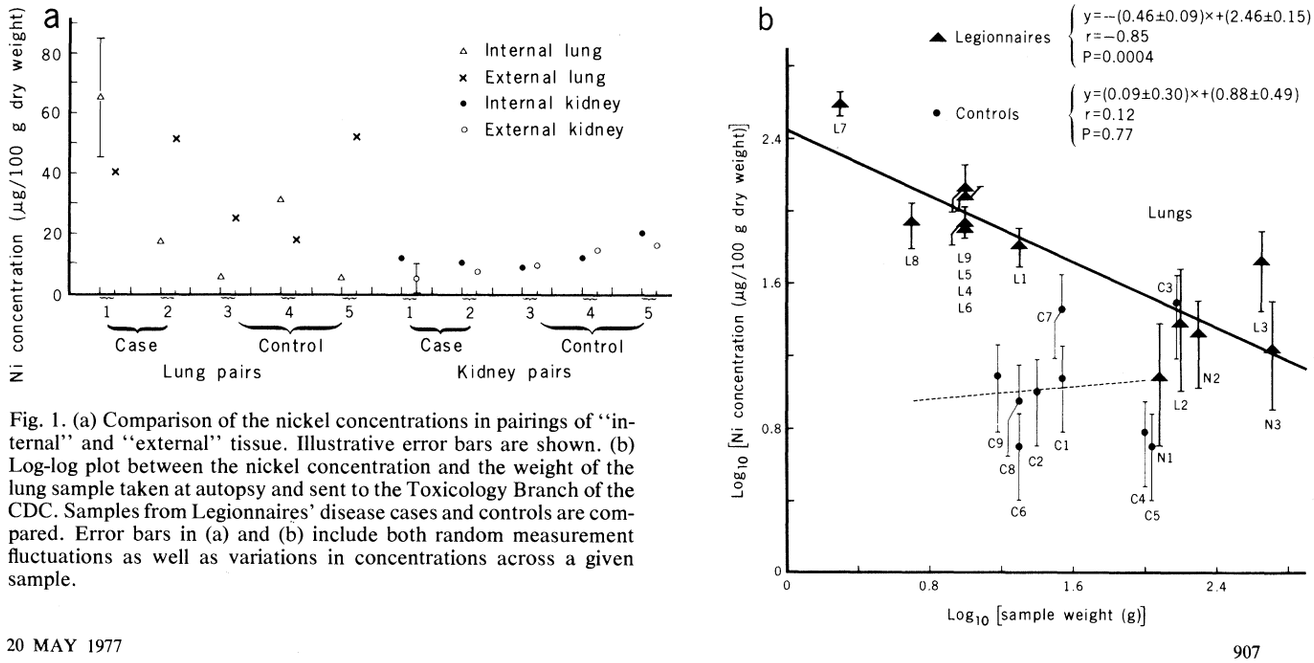

Fig. 1. (a) Comparison of the nickel concentrations in pairings of "internal" and "external" tissue. Illustrative error bars are shown. (b) Log-log plot between the nickel concentration and the weight of the lung sample taken at autopsy and sent to the Toxicology Branch of the CDC. Samples from Legionnaires' disease cases and controls are compared. Error bars in (a) and (b) include both random measurement fluctuations as well as variations in concentrations across a given sample.

"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