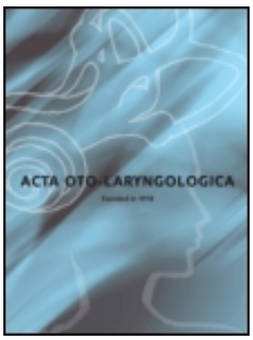

Acta Oto-Laryngologica

ISSN: 0001-6489 (Print) 1651-2251 (Online) Journal homepage: http://www.tandfonline.com/loi/ioto20

Changes in Human Nasal Mucosa during Experimental Coronavirus Common Colds

Mats Bende, Ian Barrow, Julia Heptonstall, Peter G. Higgins, Widad Al-Nakib, David A. J. Tyrrell & Anders Åkerlund

To cite this article: Mats Bende, Ian Barrow, Julia Heptonstall, Peter G. Higgins, Widad Al-Nakib, David A. J. Tyrrell & Anders Åkerlund (1989) Changes in Human Nasal Mucosa during Experimental Coronavirus Common Colds, Acta Oto-Laryngologica, 107:3-4, 262-269, DOI: 10.3109/00016488909127507

To link to this article: http://dx.doi.org/10.3109/00016488909127507

Published online: 08 Jul 2009.

Acta Otolaryngol (Stockh) 1989; 107: 262–269

Changes in Human Nasal Mucosa during Experimental Coronavirus Common Colds

MATS BENDE,[1] IAN BARROW,[2] JULIA HEPTONSTALL,[2] PETER G. HIGGINS,[2] WIDAD AL-NAKIB,[2] DAVID A. J. TYRRELL[2] and ANDERS ÅKERLUND[1]

From the [1]Department of Otorhinolaryngology, Skövde, Sweden, and the [2]Medical Research Council, Common Cold Unit, Harvard Hospital, Salisbury, Wiltshire, England

Bende M, Barrow GI, Heptonstall J, *et al.* Changes in human nasal mucosa during experimental coronavirus common colds. Acta Otolaryngol (Stockh) 1989; 107: 262–269.

Twenty-four adult volunteers were inoculated with nasal drops containing a coronavirus of 229E serotype to determine the differences in the clinical and physiological reactions which occur between clinically infected, sub-clinically infected and non-infected individuals. Thirteen volunteers were clinically infected, 8 had sub-clinical infections and 3 were uninfected. Nasal airway resistance and the temperature of the nasal mucosa increased in all infected subjects both with and without symptoms: the core temperature increased also but to a lesser extent. Mucosal blood flow and nasal secretion increased only in those with symptoms. The albumin content of the nasal secretion increased in the clinically infected, suggesting that it was derived, partially at least, from the circulation. The nasal cycle of variation in airway resistance between the two sides of the nose was observed in all three groups but increased only in those clinically infected. *Key words: coronavirus, common cold, nasal airway resistance, nasal mucosa, blood flow, nasal temperature, secretion, albumin, lactoferrin, nasal cycle.*

M. Bende, Department of Otorhinolaryngology, Central Hospital, S-541 85 Skövde, Sweden.

The common cold is one of the most frequent diseases of Man and is therefore of social and economic importance. No antiviral treatment suitable for general clinical use is available, although there are some interesting developments at the research stage (1). It is consequently worthwhile to seek treatments that block the inflammation and increase in secretion which are the hallmarks of this disease. However, we need more detailed knowledge of the pathogenesis and pathophysiology if more rational methods of managing the disease are to be developed.

The symptoms which characterize the common cold result from inflammation of the nasal mucosa and are likely to be associated with changes in its vascular bed. Although some infected volunteers suffer nasal congestion and discharge, an equal number of infected subjects have no definite symptoms and we do not know the reason for the differences in their responses.

The purpose of the present study was to evaluate the pathophysiology of a common cold in a controlled manner particularly as regards the reactions of the vascular bed. We wished to study nasal blood flow, nasal airway resistance, nasal and central body temperature, nasal secretion and vascular permeability in volunteers challenged with a coronavirus, and to correlate the changes observed to the presence or absence of infection and clinical symptoms.

MATERIALS AND METHODS

This investigation was performed at the MRC Common Cold Unit (CCU), Salisbury, England, during March and April, 1987. The experiments were approved by the Harrow

District Ethical Committee, Northwick Park Hospital, Middlesex. Twenty-four volunteers (9 men and 15 women; mean age 41.1 years, range 21.9–53.9) were isolated in groups of 2 or 3 in separate living accomodation. Of them, 17 were non-smokers, 5 smoked more than 10 cigarettes per day and 2 smoked occasionally. All were without pronounced septal deviation and had normal findings at anterior rhinoscopy. After 2 days in isolation, the volunteers were inoculated with approximately 10^2 $TCID_{50}$ of human coronavirus, strain LP (229E serotype) as nasal drops.

Clinical evaluation

The clinical effects of challenge were monitored by an observer who was unaware of the details of the study, and each volunteer was given a daily score. The score was based on symptoms and signs such as sneezing, nasal stuffiness, sore throat, headache, nasal discharge and the number of handkerchiefs used (2). Every morning and evening the volunteers measured the temperature in the mouth. At the end of the trial volunteers were graded, by the clinical observer, as having no cold, or a mild, moderate, or severe cold.

Virological tests

Nasal washes were collected daily for virus isolation. About 3 weeks after inoculation, blood was collected for convalescent serum. Virus was isolated by inoculation of roller tube cultures of C6 cells (3) and antibodies were detected by a standard neutralization test. These investigations were performed blind without knowledge of clinical assessment and from them it was possible to detect sub-clinical infections in those volunteers who did not have a cold clinically. The criteria of infection were a $\geq$4-fold rise in antibody titre and/or virus excretion on any day. Thus, finally the volunteers were divided into three groups: clinically infected, sub-clinical infection, and uninfected.

Physiological measurements

These were made at the same time of day on six occasions during the trial. The initial measurements were performed on the second day of isolation (day -1) before challenge with virus. After one day free, the day of challenge, the measurements were repeated for 5 days (day 1 to day 5) using the methods given below.

Congestion

Nasal airway resistance to airflow (NAR) was measured bilaterally (after 30 min at rest and acclimatization to indoor conditions) using anterior rhinomanometry (rhinomanometer no. 1, Mercury Electronics Ltd, UK) and calculated for the total nose using a computer and the method of Broms et al. (4). NAR was expressed as V_2, and increasing values of V_2 denoted increasing NAR.

Temperature

Nasal mucosal temperature was recorded on the surface of the inferior turbinate bilaterally by an isolated thermistor (thermometer CTC 85, probe A-E5, Ellab, Denmark) in accordance with a recently described method (5). Mean nasal mucosal temperature was the mean temperature of right and left side.

The central body temperature was recorded with the probe placed close to the left ear drum under visual guidance with an otoscope. This site was chosen as it gives a more accurate estimate than that recorded orally. A thermistor identical to that used for measuring the nasal temperature but without insulation was employed.

Blood flow

Nasal mucosal blood flow was recorded in the supine position in the left nostril by the ^{133}Xe washout method (6). With this method 0.1 ml of xenon in saline was deposited in the nasal mucosa of the inferior turbinate. The disappearance of the isotope was monitored using a scintillation detector placed over the nose. The blood flow was calculated from the slope of the washout curve and expressed in ml/min/100 g tissue. This method assesses the mean perfusion of the nasal mucosa.

Secretion

Paper handkerchiefs used by the volunteers were weighed to provide an estimation of the total daily nasal secretion. Nasal secretion was also investigated qualitatively by the nasal spray washing method of Linder et al. (7). The two nasal cavities were sprayed repeatedly with a saline solution containing lithium chloride (1 mM) as an exogenous marker and the volunteers expelled the fluid into a plastic dish. The fluid was filtered through a 5 μm Millipore filter and the filtrate was frozen to −20°C pending assay. The samples were analysed for albumin and lactoferrin by a radial immunodiffusion technique in order to determine the source of the secretion. The dilution factor of the lithium was used to calculate the concentration of albumin and lactoferrin. However, the accuracy of the test varies with the amount of these substances present, so that with little secretion, estimates are only approximate.

Statistics

The area under the curve (AUC) was used for the statistical analyses of the effect of virus inoculation. The calculations were performed with paired *t*-test for data of NAR, temperature and blood flow, and with Wilcoxon rank sum test for the results of secretion, albumin and lactoferrin tests. Correlation tests were performed with a non-parametric test, Spearman rank order correlation coefficient. Results are expressed as mean ±SE.

RESULTS

Of 24 volunteers challenged with virus, 13 developed significant clinical signs of a common cold, 8 had sub-clinical infections and 3 remained uninfected (Table I). Of the 13 colds 11 were classified as mild and two as moderate. The mean daily scores are presented for the different groups in Fig. 1 and total scores in Table I. There was an excess of women and smokers in the group of volunteers who had colds, but this was not unexpected (8).

The effect of a common cold

The initial NAR for all volunteers was 37.4±2.3. The change in NAR during the trial is plotted in Fig. 2. NAR increased by about 40% in both the clinically infected group

Table I. *Effect of challenge in relation to sex, smoking habits, age and total clinical scores*

	n	Sex		Smoker		Age (years±SD)	Total score mean (range)
		m	f	−	+		
Cold	13	2	11	8	5	40.1±9.6	25.4 (8–58)
Sub. inf.	8	7	1	8	0	43.2±7.9	1.3 (0–3)
No inf.	3	0	3	1	2	40.3±9.2	1.3 (0–2.5)

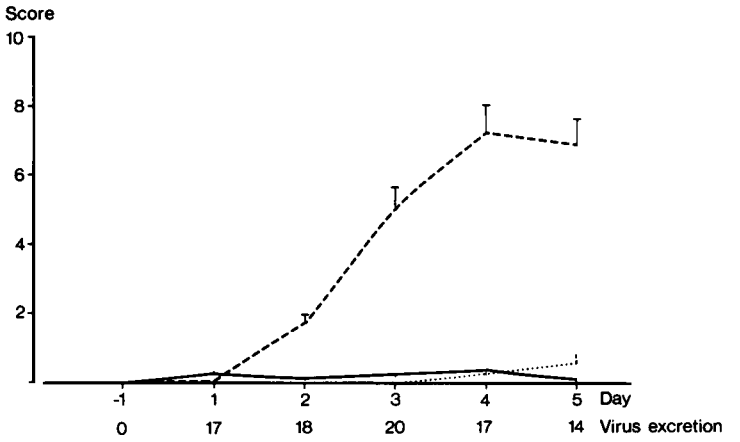

Fig. 1. The mean daily clinical scores of volunteers with clinical infections (– – –), sub-clinical infections (——) or no infection (· · · ·). The number of volunteers who excreted virus each day is indicated below.

($p<0.01$) and in those sub-clinically infected ($p<0.05$) compared with the initial day (AUC analyses, paired t-test). There was a trend to increased NAR in the uninfected subjects but values were below those of infected subjects and statistically significantly so by the end of the observation period.

The mean nasal mucosal temperature of all volunteers was 30.1±0.3°C before inoculation but the group which successfully resisted infection had lower temperatures than those who became infected. There was a statistically significant increase in nasal temperature in both the clinically infected ($p<0.001$) and the sub-clinically infected groups. ($p<0.01$) (Fig. 3). On day 3 the mean nasal mucosal temperature in the volunteers with clinical infections was 33.2±0.3°C. Though there was a rise in the uninfected group the temperature was below that of the other two groups by the end of the trial.

Mean ear temperature of all volunteers was 36.2±0.1°C initially. This increased significantly ($p<0.05$) in both the clinically infected and the sub-clinically infected groups. On

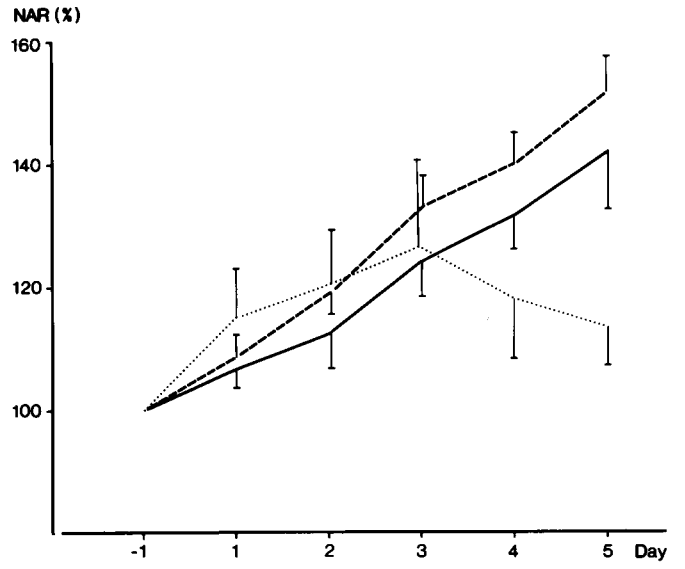

Fig. 2. The change in mean nasal airway resistance to airflow in the three groups, clinical infections (– – –), sub-clinical infections (——) and no infection (· · · ·).

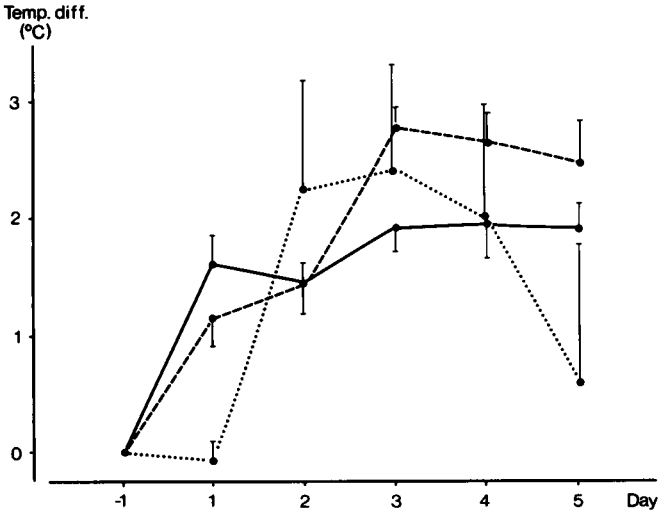

Fig. 3. The change in mean daily nasal mucosal temperature in volunteers with clinical infections (– – –), sub-clinical infections (——) and no infection (· · · ·).

day 3 the ear temperature in the 'cold' group was 36.7±0.1°C. The maximum increase in mean ear temperature was about 0.5°C in the clinically infected group on day 3.

The initial measurement of blood flow of the nasal mucosa of all volunteers was 43.4±2.0 ml/min/100 g. In Fig. 4 the change in blood flow of each group is plotted against time. The blood flow increased by over 20% ($p<0.05$) in the clinically infected group but not in the others. Blood flow values were missing for 6 volunteers on day 5.

Secretion, as measured by weighed handkerchiefs, was significantly increased ($p<0.01$) only in the clinically infected group (Fig. 5). The minimum amount of secretion in the other groups made the determinations of albumin and lactoferrin concentrations too inaccurate to justify calculation. In the clinically infected group the concentration of albumin in secretion was significantly elevated ($p<0.05$), from approximately 730 mg/l pre-challenge to a maximum of 2996±1132 mg/l on day 5, but there was no significant change in that of lactoferrin, which remained at below 700 mg/l (AUC Wilcoxon test).

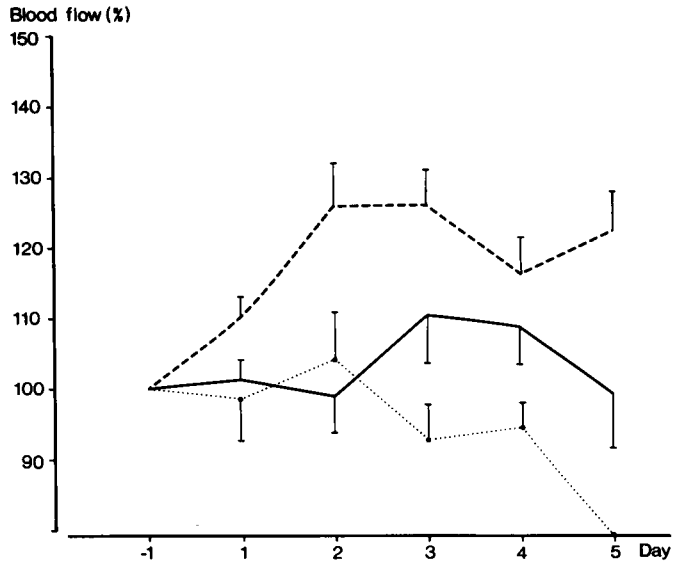

Fig. 4. The change in mean nasal mucosal blood flow in volunteers with clinical infections (– – –), sub-clinical infections (——) and no infection (· · · ·).

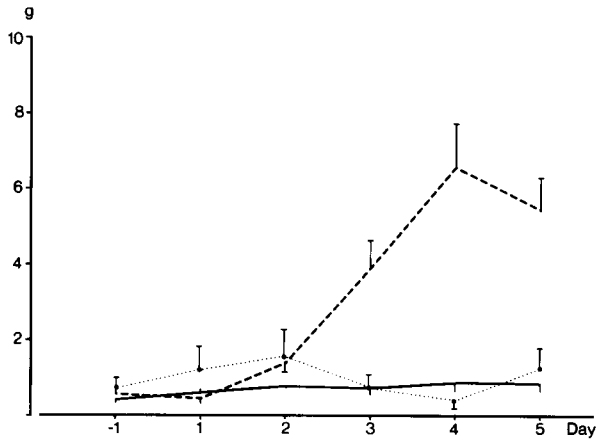

Fig. 5. The mean daily amount of secretion, measured by weighing tissues, from the groups with clinical infections (---), subclinical infections (——) and no infection (····).

Differences in NAR between the two sides of the nose are frequently observed. These may be constant, as in those with anatomical abnormalities, e.g. deviated septum, or vary from time to time, in line with the thickness of the nasal mucosa, and occur in 80% of normal individuals. This nasal cycle was observed in all three groups of volunteers but increased only in the clinically infected subjects (Table II) reaching a maximum on day 5 when the difference from the pre-challenge value was significant ($p<0.01$).

On the first day after inoculation the only effect parameter that had changed significantly ($p<0.05$) compared with the initial value at day −1 was mean nasal temperature and this occurred in all infected volunteers.

Table II. *Differences in NAR between the nasal cavities during the study in volunteers who got a cold (n = 13)*

Day	NAR difference
−1	14.4±3.5
1	20.7±4.8
2	17.6±3.1
3	23.5±2.6
4	27.7±5.1
5	31.5±4.2

Table III. *Spearman rank order correlation coefficients between the indicators of infection and the physiological measurements*

	Total score	Titre change	Virus excretion
NAR	0.36*	0.35*	0.15
Nasal temperature	0.14	0.14	0.01
Ear temperature	−0.03	−0.02	0.06
Blood flow	0.37*	0.45*	0.49*
Secretion	0.68***	0.09	0.27

*$p<0.05$, ***$p<0.001$.

Correlations

The parameters NAR, mean nasal temperature, ear temperature, nasal mucosal blood flow and secretion were correlated to 1) the total score, 2) the fold increase in the specific coronavirus antibody titre, and 3) the number of days of virus excretion. The correlations are presented in Table III.

DISCUSSION

By studying experimental colds we were able to make more comprehensive observations on our volunteers than is possible in patients seeking medical advice. It should be realised that the illnesses were mild, with only 0.5°C rise in temperature and few systemic symptoms. They more closely resemble the illnesses in the home than those that come to medical attention. Furthermore, we studied only infections caused by a coronavirus—these viruses are responsible for perhaps one cold in five, though clinically they are very similar to colds caused by rhinoviruses, respiratory syncytial virus, etc.

From our study we can conclude that coronavirus colds are associated with increased NAR, mucosal temperature and blood flow in the nose. It is of interest that the first two of these were also increased in sub-clinical infections. The distinguishing feature between clinical and sub-clinical infection is an increase in nasal secretion and suggests that treatment should, if possible, block secretion without interfering with the increase in circulation or rise in mucosal temperature (see below).

It seems at first contradictory that there should be an increase in nasal temperature in subjects who were classified, eventually, as not infected. However, we have some evidence that there may be a short local immune response and may indeed be a brief occult cycle of virus multiplication (Callow, unpublished), and, if so, it is at least possible that this is sufficient to induce a brief physiological change resulting in a rise in temperature. However, the present observation is based on only 3 subjects and is worth further investigation.

It is known that the temperature sensitivity of the replicative cycle is an important element in the virulence of a virus. Furthermore experiments on various animal species infected with viruses ranging from poliovirus to influenza have shown that hyperthermia decreases the severity of viral infections, whereas hypothermia increases it (9–13). The viruses which are often associated with the common cold, such as rhinoviruses and coronaviruses (14), are all temperature sensitive and replicate optimally only at temperatures below the core temperature (15). Because of the cooling produced by the air flow, the temperature of the nasal mucosa is normally a few degrees lower than the central body temperature, and this provides suitable conditions for their replication. It is, therefore, interesting that the nasal temperature was the physiological parameter that reacted first in the study and also that it increased much more than the body temperature in both clinically and sub-clinically infected volunteers. These observations suggest that a rise in temperature may be the first line of defence against coronavirus infections.

Viruses in general induce the production of small molecular weight proteins, previously known as endogenous pyrogen, which in turn activate various immunologically active phagocytic cells. These pyrogens may also act on the thermoregulatory centre in the hypothalmus leading to fever (16, 17). Small elevations in body temperature, like those seen in this study, can improve the action of leukocytes, lymphocytes and interferon. Fever may, therefore, not be just a by-product of disease (18–20) but have wider beneficial effects in infection than the simple inhibition of virus replication.

Although nasal mucosal temperature increased significantly in all volunteers infected with virus, there were no correlations with the measures of infection (Table III). From a

previous study (5) we know that changes in mucosal temperature do not correlate with changes in NAR. Nasal mucosal blood flow, NAR and secretion, on the other hand, correlated significantly with the severity of symptoms, as measured by the clinical score (Table III).

The increase in secretion in a coronavirus cold starts later than other pathophysiological parameters as seen in Figs. 2–5 and our study was designed to reveal the source of this secretion. The increase in the concentration of albumin in the nasal secretion indicated that there was at least some leakage from the blood circulation and the absence of an increase in the concentration of lactoferrin suggests that the seromucous glands in the nasal mucosa made only a small contribution. Further studies would be of interest.

ACKNOWLEDGEMENTS

We are indebted to Ulla Thunberg for technical assistance, to Aud Nyholm, Margareta Bouveng and Gunnar Ronquist for analysis of the secretion and to John Crawley for help with the scintillation detector. This investigation was supported by grants from the Foundation of Torsten and Ragnar Söderberg and from LEO Ltd., Helsingborg Research Foundation.

We thank Mrs A. Dalton and Miss J. Dunning for help in clinical assessment and our technical staff for virological examinations. We are most grateful to the volunteers for their willing co-operation.

REFERENCES

1. Tyrrell DAJ, Al-Nakib W. Prophylaxis and treatment of rhinovirus infections. In: De Clercq E, ed. Clinical use of antiviral drugs. Norwell, MA: Kluwer-Nijhoff, 1988: (in press).
2. Beare AS, Reed SE. The study of antiviral compounds in volunteers. In: Oxford J, ed. Chemoprophylaxis and chemotherapy of viral infections of the respiratory tract, vol 2. Cleveland, Ohio: CRC Press, 1977; 27–55.
3. Phillpotts RJ. Clones of MRC-C cells may be superior to the parent line for the culture of 229E like strains of human respiratory coronavirus. J Virol Methods 1983; 6: 267–9.
4. Broms P, Johnson B, Lamm CJ. Rhinomanometry. A system for numerical description of the nasal airway resistance. Acta Otolaryngol (Stockh) 1982; 94: 157–68.
5. Åkerlund A, Bende M. Nasal Mucosal temperature and the effect of common cold. Clin Otolaryngol 1988.
6. Bende M, Flisberg K, Larsson I, Ohlin P, Olsson P. A method for determination of blood flow with ^{133}Xe in human nasal mucosa. Acta Otolaryngol (Stockh) 1983; 96: 277–85.
7. Linder A, Ronquist G, Deuschl H. Random distribution of exogenous lithium in nasal secretion and its application in substance determination. Acta Otolaryngol (Stockh) 1983; 287–93.
8. Callow KA. Effect of specific humoral immunity and some non-specific factors on resistance of volunteers to respiratory coronavirus infection. J Hyg Camb, 1985; 95: 173–89.
9. Armstrong C. Some recent research in the field of neurotropic viruses with especial reference to lymphocytic choriomeningitis and herpes simplex. Military Surgeon 1942; 91: 129–45.
10. Lwoff A. Factors influencing the evolution of viral diseases at the cellular level and in the organism. Bact Rev 1959; 23: 109–24.
11. Lwoff A. Death and transfiguration of a problem. Bact Rev 1969; 33: 390–403.
12. Kirn A, Schieffer K, Braunwald J. L'hyperthermie provoquée au cours de l'encéphalite a virus vaccinal de la souris. Annales de l'institut Pasteur 1966; 111: 647–54.
13. Toms GL, Davies JA, Woodward CG, Sweet C, Smith H. The relation of pyrexia and nasal inflammatory response to virus levels in nasal washings of ferrets infected with influenza viruses of differing virulence. Br J Exp Path 1977; 58: 444–58.
14. Larson HE, Reed SE, Tyrrell DAJ. Isolation of rhinoviruses and coronaviruses from 38 colds in adults. J Med Virol 1980; 5: 221–9.
15. Tyrrell DAJ. Rhinoviruses and coronaviruses—virological aspects of their role in causing colds in man. Eur J Respir Dis 1983; 64: 332–5.
16. Atkins E, Bodel P. Fever. New Engl J Med 1972; 286: 27–34.
17. Dinarello CA, Wolff SM. Pathogenesis of fever in man. New Engl J Med 1978; 298: 607–12.
18. Stern RC. Pathophysiologic basis for symptomatic treatment of fever. Pediatrics 1977; 59: 92–8.
19. Keusch GT. Fever; to be or not to be. N Y State J Med 1976; 76: 1998–2001.
20. Kluger MJ. Fever. Pediatrics 1980; 66: 720–4.