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3256

Tear Fluid Electrolytes and Albumin in Persons under Environmental Stress

J. E. M. THYGESEN,* B. BACH,† L. MØLHAVE,† O. F. PEDERSEN,† J. U. PRAUSE,* AND P. SKOV‡

**Eye Pathology Institute, University of Copenhagen, Frederik V's Vej 11, 2100 Copenhagen Ø, †Institute of Hygiene, Universitetsparken, Bygn. 180, 8000 Århus C, and ‡Department of Occupational Medicine, Rigshopitalet, University of Copenhagen, Blegdamsvej 9, Copenhagen Ø, Denmark*

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Sixty-two subjects selected among 287 persons with indoor air complaints were exposed to a standard mixture of 22 different organic gases and vapors normally found in Danish houses. Persons were randomly assigned to one of four exposure groups, and each subject stayed during the test day from 10:00 AM to 4:00 PM under standardized conditions in a climate chamber. During exposure the blink frequence was recorded, and after exposure the tear fluid contents of serum albumin, potassium, and sodium were measured. It was found that the persons had an increased concentration of serum albumin in the tear fluid, and that exposure to high concentrations of organic gases and vapors induced a tear reflex-mediated dilution of the tears.

INTRODUCTION

One of the consequences of the oil crisis has been a change in building construction in the western countries. The living quarters are now well insulated and draughtproof. Despite efforts to condition the climate of the new buildings, complaints of dry mouth and eyes, headaches, and conjunctivitis related to indoor climate present a growing problem. In WHO, the Euro Report, a "sick building syndrome" has been defined (WHO, 1983). Vapors of organic solvents and formaldehyde from modern construction material (Mølhave, 1982) have been suspected possible etiologies.

To evaluate the influence of such gases and vapors on the feeling of well being of test subjects known to have complaints related to indoor air in homes or in workplaces, a carefully controlled masked study was initiated in which the test persons were exposed to a standardized mixture of 22 organic gases and vapors in a climate chamber (Mølhave *et al.*, 1984). As a part of this study eye reactions were also examined, using blinking frequence and tear fluid concentrations of Na^+, K^+, and serum albumin (s-A) as parameters.

MATERIALS AND METHODS

The investigation was undertaken in the climate chambers of the Institute of Hygiene, Århus, Denmark. The selection of subjects for the exposure is described in detail elsewhere (Mølhave *et al.*, 1984). In the exposure experiment, 62 subjects (39 females, 23 males, average age 38.9 years) were selected among 287 persons with indoor air complaints. All subjects passed a general medical exami-

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nation to ensure that no one suffered from systemic or local diseases before entering the study.

The subjects being exposed during only 1 day were randomly assigned to one of four exposure groups (Table 1). All subjects were exposed to a standardized mixture of 22 different organic gases and vapors normally found in Danish houses (Mølhave *et al.*, 1984) (Table 2). Each subject stayed during the test day from 10:00 AM to 4:00 PM under standardized conditions in a climate chamber. The day was divided in two exposure periods, each of 2¾ hr duration. Each subject was exposed to either 5 or 25 mg/m^3 of the vapor mixture in one of the two periods, and to clean air in the other. These concentrations correspond to the average and the maximum found in new Danish homes. Thus four exposure groups were established (Table 1); two of these groups were exposed during the morning, while the other two were exposed in the afternoon.

Several subjective and objective measurements were performed during exposure. This study describes measurements of the blinking frequency together with Na^+, K^+, and s-A concentrations in tear fluid. Other publications describe mental subjective and mucous membrane reactions (Mølhave *et al.*, 1985) and performance and subjective skin reactions (Bach *et al.*, 1984) during exposure.

The eyes were inspected before and after exposure, and the blinking frequency was recorded during each exposure period, by use of a video recorder.

Tear samples were collected from all subjects immediately after completion of the total exposure period in the chamber, by use of cellulose sponges (4 × 4 × 4 mm) placed in the lower fornix of the eye. Tear-filled sponges containing approximately 10 µl fluid were stored in microcentrifuge tubes at −20°C.

Tear fluid was separated from the sponges by centrifugation (2500 g/5 min). The content of s-A was determined using the microelectroimmunoassay (Prause *et al.*, 1978). The contents of sodium and potassium were determined by flame photometry.

Differences between concentrations of electrolytes and s-A in tear fluid of persons from different groups were tested using the Mann–Whitney test, and a level of $P < 0.05$ was considered significant.

In the statistical analysis two hypotheses were tested. First, was there a short-term exposure effect causing the concentrations measured among the afternoon-exposed groups to be different from those among the morning-exposed groups? If so, was the effect stronger among group I (25 mg/m^3) than among group III (5 mg/m^3), thus indicating a dose–effect relationship? Second, did our groups of

TABLE I

Group	Concentration of vapor mixture (mg/m^3)	
	10:00 AM–1:00 PM	1:00–4:00 PM
I	0	50
II	50	0
III	0	10
IV	10	0

TABLE 2
COMPOSITION OF STANDARDIZED VAPOR MIXTURE

Chemical	Weight part of total (30.91 parts)
n-Hexane (Merck art. 4367)	1
n-Nonane (Fluka 74252)	1
n-Decane (BDH produkt 28001)	1
n-Undecane (BDH produkt 15066)	0.1
1-Octene (Fluka 74903)	0.01
1-Decene (Fluka 30650)	1
Cyclohexane (Merck art. 9666)	0.1
3-Xylene (Fluka 95672)	10
Ethylbenzene (Merck art. 801372)	1
1,2,4-Trimethylbenzene (Fluka 797445)	0.1
n-Propylbenzene (Merck art. 807521)	0.1
α-Pinene (Kock–Light 67703)	1
n-Pentanol (Fluka 94512 (58211)	0.1
n-Hexanol (Merck art. 802672)	1
Isopropanol (Merck. art. 9634)	0.1
n-Butanol (Merck art. 1990)	1
2-Butanone (Merck art. 9708)	0.1
3-Methyl-3-butanone (Fluka 59600)	0.1
4-Methyl-2-pentanon (Merck art. 820820)	0.1
n-Butylacetate (Merck art. 9652)	10
Ethoxyethylacetate (Kodak p. 2378)	1
1,2-Dichloroethane (Fluka 155831)	1

selected subjects, who themselves claimed to be sensitive to indoor air quality, differ from the normal population? Regarding blinking frequency, the hypothesis was that the exposure would increase the frequency due to eye irritation.

RESULTS

The blinking frequencies were not normally distributed, but this was the case for the differences between control and exposure periods. A variance analysis of differences therefore was performed with age, sex, smoking habits, and time of day as dependent variables. No significant effect of exposure was found, but the very large individual variation in blinking frequency might have obscured any small effects. No correlation was found between blinking frequency and other parameters related to eye irritation. For technical reasons, samples of tear fluid were not taken during the first 4 exposure days. Therefore, only 48 subjects were examined. The samples in this case were taken at 30 to 60 min after the last exposure period. Table 3 shows the results.

Tear s-A concentrations were not normally distributed. Tables (2 × 2) were then tested by a χ^2 test as shown in Tables 4 and 5. A significant effect and a tendency to effect (0.05 cpc 0.10) of exposure were found, when the subjects were divided according to arithmetic mean and median albumin concentration, respectively. No exposure differences were found between morning and afternoon results.

TABLE 3

Group	Tear serum albumin median, range (μg/ml)	Tear K^+ median, range (mmoles/liter)	Tear Na^+ median, range (mmoles/liter)
I	20.8 4.4–85.7	2.6 0.9–48.5	39 13–234
II	27.4 4.8–88.2	3.3 0.9–58.9	39 13–251
III	36.9 7.4–125*	4.3 2.2–18.2	52 30–108
IV	74.8 9.6–125*	11.3 3.0–50.3	87 26–225

Note. Serum albumin: I = II, I < III ($P < 0.05$), I < IV ($P > 0.01$). II < IV ($P < 0.01$), III < IV ($P < 0.01$); K^+: I < III, II < IV ($P < 0.05$), I < IV ($P < 0.01$); Na^+: I < IV ($P < 0.05$).

* Concentrations higher than 125 μg/ml were recorded as 125 μg/ml.

In testing for difference between the single groups by means of the Wilcoxon test, as seen in Table 3, the subjects from group I have statistically significant lower concentrations of tear s-A than do persons from groups III and IV, but the same concentrations as subjects from group II.

Subjects from group II also differed significantly from those from groups III and IV, and subjectgs from group III had significantly lower s-A in tear fluid than did persons from group IV (Table 3).

The Na/K ratios were approximately normally distributed, but no effect of dose could be found either when the four exposure groups were compared mutually or when morning-exposed persons were compared with afternoon-exposed persons.

DISCUSSION

Weber and Tschapp (1977) measured the threshold limit of formaldehyde vapor with regard to influence of blinking frequency, and found it to be 1.7 ppm. Formaldehyde is much more irritating than are any of the compounds used in this experiment. This may explain why we found no effect.

Unfortunately no proper reference measurements exist. The signal to noise ratio in the present experiments is low and a high detection limit is expected. Irritation does not, however, always lead to increased blinking frequency (Schuck *et al.*, 1966).

In recent studies it has been shown that patients with inflammatory reactions in the cornea have raised concentrations of s-A in the tear fluid (Liotet *et al.*, 1979; Prause, 1983). Patients wearing contact lenses causing even minor discomfort have increased leak of serumproteins to the tear fluid (Liotet *et al.*, 1979; Lundh *et al.*, 1984). The present study shows that subjects complaining of eye discomfort and exposed to irritating vapors have increased tear fluid concentrations of

TABLE 4

RESULTS OF ALBUMIN ANALYSIS: χ^2 TEST ACCORDING TO MEDIAN ($\bar{x}$)

	Number of results $< \bar{x}$	Number of results $> \bar{x}$	
Doses 25 mg/m³	13	4	$P \sim 0.5\%$
Doses 5 mg/m³	6	14	

TABLE 5
RESULTS OF ALBUMIN ANALYSIS: χ^2 TEST ACCORDING TO ARITHMETIC MEAN (x)

	Number of results $< \bar{x}$	Number of results $> \bar{x}$	
Doses 25 mg/m³	13	4	$P \sim 10\%$
Doses 5 mg/m³	11	11	

s-A. Despite the rather crude collection method in the present study using cellulose sponges, which by themselves may induce leakage of serumproteins from the conjunctival vessels, the findings are significant.

A dose-related response seems to exist because subjects immediately after exposure to the high concentrations (25 mg/m³) of organic gases and vapors (group I) had a tear fluid concentration of s-A close to normal, while subjects immediately after exposure to the lowest concentration (group III) or some time later (group IV) had the highest s-A concentration. This might be explained by an increased stimulation of the trigeminal nerve tear reflex (group I), so that the concentration of s-A in the tear fluid would fall because of dilution. The present study design did not allow collection of tear fluid prior to exposure, and the basic tear fluid concentration of s-A was not measured.

Compared with the normal concentration of s-A in tear fluid of 10.3 ± 9.2 μg/ml (±2 μg/ml SD), one-half the subjects from group I fell within the limits, while only three of groups II and III and one of group IV were normal.

The same pattern of changes in tear fluid electrolytes could be demonstrated (Table 3), although not as marked.

Our finding of increased concentrations of potassium and sodium in the tear fluid compared with normal standards, and the tendency to normalization after the high doses, supports the hypothesis.

In conclusion, exposure to a mixture of 22 organic gases and vapors of the kind and concentrations found in the room air of newly built homes seems to affect the tear fluid. The tear content of serum albumin is increased due to an increased leak from conjunctival vessels with a dilution effect immediately after heavy exposition. This irritation did not seem to influence the frequency of eye blinking.

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