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# CHANGES IN HUMAN ORAL MICROBIOTA AND LOCAL IMMUNITY UNDER ARTIFICIAL CONDITIONS

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***SUMMARY***
*The article shows the role of extreme influences on the development of pathological changes in the dento-mandibular system. The possibility of developing a system of preventive measures non-biocidal action, mainly using probiotics, in particular autoprobiotics is considered.*

***KEYWORDS****: modified environment, oral microbiocinosis, and local humoral immunity.*

***CONFLICT OF INTEREST.*** *The authors declare no conflict of interest.*

## Introduction

Habitat can be changed artificially. This happens when a person, driven by a scientific interest tries to know the world around him. This acquires the greatest significance in the conditions of space exploration, the sea depths of the earth's interior. To study these hard-to-reach areas in everyday life, artificial anthropoecosystems with modified habitat parameters are being created. In such changed conditions, the phylogenetically established relationships of the co-actants of the ecological system "man-microorganisms" undergo changes. It takes the form of disordered colonization resistance syndrome [1]. Bacteria are the predominant microorganisms in the resident oral microbiota. The resident microbiota of the oral cavity, the main part of which is formed by bacteria, competes with exogenous pathogens and defeats them, and also contributes to the normal development of tissues and the

immune system. However, the homeostatic balance between the host and microbial communities can be disturbed by many factors that can lead to the development of oral diseases such as caries, gingivitis, periodontitis, pharyngotonsillitis, etc.

**The aim** of the work was to characterise the three main barriers to infection that form in humans: defence group microflora, covering tissues and immunity, in conditions of isolation in hermetically sealed objects with altered environments in long-duration diving, in conditions simulating several factors of space flight (isolation and hypokinesia) and in spaceflight conditions.

## Materials and methods

The determination of immunoglobulins in saliva was carried out using the nephelometric method. The content of immunoglobulins was studied in mixed unstimulated saliva obtained before meals. Monoclonal antibody solutions against sIgA, IgA, IgG antigens were used to determine immunoglobulin concentration. The traditional method of bacteriological analysis was used to identify microorganisms. Bacteriological analysis was performed by seeding on nutrient media (HiCrome Candida Agar, Staphylococcus Agar No10, Columbia Blood Agar Base, Columbia Blood Agar Base + Staph Strepto Supplement, Columbia Blood Agar +Non Spore Anaerobic Supplement).

## Results and discussion

*Insulation in hyperbaric (up to 40 atmospheres) containment facilities*

Under hyperbaric conditions, practically all biotopes, including the periodontal region, become involved in the pathogenization of microflora [2, 3]. There is a quantitative reduction of non-pathogenic protective groups of microorganisms and a quantitative increase in opportunistic ones. Various microorganisms are involved in this process, regardless of their tinctorial properties, relationship to air oxygen, and taxonomic characteristics. A negative correlation between Veilonellae (protective group) and Actinomycetes (causative agents of periodontitis) can be observed (*Table 1*).

*Table 1*

***Quantitative characterisation of Veilonellae and Actinomycetes periodontalis in deep-sea divers***

| Operator, micro-organism groups | A 24-hour long dive | | | |
|---|---|---|---|---|
| | 0 | 3 | 6 | 10 |
| ***Veilonella sp.*** | 7 | 5 | 0 | 0 |
| ***Actinomyces naeslundii*** | 0 | 7 | 7 | 6 |
| ***Veilonella sp.*** | 5 | 5 | 5 | 5 |
| ***Actinomyces naeslundii*** | 0 | 0 | 7 | 5 |
| ***Veilonella sp.*** | 5 | 5 | 5 | 5 |
| ***Actinomyces naeslundii*** | 0 | 0 | 0 | 0 |

The gingival sulcus sediment prior to immersion was found to contain resident microflora consistent with normal. During the experiment an increase in the number of bacteria capable of supporting the inflammatory process and the appearance of parodontopathogenic bacteria *Prevotella melaninogenica, Actinomices israelii, Actinomices naeslundii and Fusobacterium nucleatum* against the background of the disappearance of bacterial groups *(Veillonella spp., Str. salivarius*) constituting normal oral microbiocenosis was shown. The content of immunoglobulins before immersion usually corresponded to the lower limit of normal. During the experiment the number of immunoglobulins increased significantly, which seems to reflect the sensitization of tissues by oral antigens, mainly toxins of microorganisms.

*Table 2*

***Qualitative and quantitative composition of the gingival sulcus microflora in subjects under prolonged submersion by saturation***

| Identified species | Study time | | | | |
|---|---|---|---|---|---|
| | Before the dive | Between the dives | The third day of long diving | End of long diving | 30 days after long diving |
| ***Subject 1*** | | | | | |
| *Peptostreptococcus anaerobius* | 8 | 9 | 9 | 6 | 6 |
| *Veillonella.spp* | 7 | 5 | - | - | 6 |
| *Streptococcus salivarius* | 7 | 5 | 5 | - | 7 |
| *S.sanguis* | 6 | 9 | 6 | 8 | 6 |
| *Actinomyces viscosus* | 6 | 9 | 8 | - | - |
| *A.naeslundii* | - | 7 | 7 | 6 | - |
| *Fusobacterium nucleatum* | - | - | 6 | - | - |
| *S.pyogenes* | - | - | 6 | - | - |
| *Prevotella melaninogenica* | - | - | - | 6 | - |
| ***Subject 2*** | | | | | |
| *Peptostreptococcus anaerobius* | 7 | 6 | 6 | 6 | 7 |
| *Veillonella.spp* | 7 | 5 | - | - | - |
| *Streptococcus. salivarius* | 7 | 5 | 5 | - | - |
| *S.sanguis* | 6 | - | - | 7 | 9 |
| *Actinomyces viscosus* | 6 | 7 | 5 | - | 7 |
| *A.naeslundii* | - | - | 5 | 5 | 7 |
| *S.pyogenes* | - | 7 | - | - | 8 |
| *Prevotella melaninogenica* | - | - | - | 6 | 8 |
| ***Subject 3*** | | | | | |
| *Peptostreptococcus apaeobius* | 7 | 9 | | 6 | 6 |
| *Veillonella spp* | 7 | 7 | | 7 | 6 |
| *Streptococcus salivarius* | 6 | 8 | | - | - |
| *S.sanguis* | 6 | 6 | | 8 | 6 |
| *Actinomyces viscosus* | 6 | - | | 6 | 6 |
| *Staphylococcus.aureus* | - | - | | - | 6 |

Table 3

**Gingival microflora of the subjects before and after their isolation in the germoobject (without hygiene recommendations and special oral hygiene products) (Lg CFU)**

| Types | Before | After |
|---|---|---|
| **Subject 1** | | |
| *S. sanguis* | 4 | 4 |
| *S. salivarius* | 3 | 3 |
| *Veillonella parvula* | - | 3 |
| *Prevotella oralis* | - | 3 |
| *P. melaninogenica* | - | 2 |
| *Fusobacterium sp.* | 2 | - |
| *Corynebacterium sp.* | 3 | - |
| *Propionibacterium sp.* | - | 3 |
| *A. naeslundii* | 2 | - |
| **Subject 2** | | |
| *S. sanguis* | 4 | 7 |
| *S. salivarius* | 3 | - |
| *P. niger* | - | 5 |
| *Veillonella parvula* | 2 | - |
| *P. melaninogenica* | - | 6 |
| *Corynebacterium sp.* | 2 | 4 |
| *Actinomyces sp.* | - | 4 |
| *Staphylococcus sp.* | - | 6 |
| *Lactobacillus sp.* | 2 | - |
| *Acinetobacter sp.* | - | 5 |
| **Subject 3** | | |
| *S. sanguis* | 5 | 3 |
| *S. salivarius* | 3 | 3 |
| *S. intermedius* | 4 | - |
| *P. anaerobius* | - | 2 |
| *Veillonella parvula* | - | 2 |
| *Prevotella oralis* | 4 | 3 |
| *P. melaninogenica* | - | 3 |
| *Lactobacillus. sp* | - | 2 |
| **Subject 4** | | |
| *S. sanguis* | 4 | 4 |
| *S. salivarius* | 2 | 3 |
| *S. intermedius* | - | 3 |
| *P. anaerobius* | - | 2 |
| *Veillonella parvula* | 2 | 2 |
| *Prevotella oralis* | 3 | - |

Table 4

**Dynamics of certain immunoglobulin concentrations during the experiment in subjects before and after their isolation in the containment facility (Mg %)**

| Immunoglobulin | Subject 1 | | Subject 2 | | Subject 3 | | Subject 4 | |
|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After |
| IgA | 5,2 | 2,0 | 6,5 | 6,0 | 7,5 | 7,5 | 7,0 | 6,0 |
| IgG | 26,0 | 36,0 | 30,5 | 61,5 | 31,0 | 36,0 | 25,0 | 30,5 |
| SIgA | 100,0 | 93,0 | 83,0 | 72,0 | 100,0 | 32,0 | 70,5 | 49,5 |

In the course of the experiment an increase in the number of bacteria capable of supporting the inflammatory process and the appearance of periodontopathogenic bacteria *Prevotella melaninogenica, Actinomices israelii, Actinomices naeslundii and Fusobacterium nucleatum* against the background of the disappearance of groups of bacteria (*Veillonella spp., Str. salivarius*) (*Table 2*) constituting the normal oral microbiocenosis was shown. The content of immunoglobulins before immersion usually corresponded to the lower limit of the norm. During the experiment the number of immunoglobulins increased significantly, which seems to reflect the sensitisation of tissues by oral antigens, mainly toxins of microorganisms.

*Long-term isolation in normobaric containment facilities*

The microflora dynamics in four subjects who were in a hermetic chamber for 110 days and used conventional hygiene products are shown below (*Table 3*).

Subject 1 showed multidirectional trends during the experiment: activation of periodontopathogens of the genus *Fusobacterium sp.,* decreased activity of Streptococcus sanguis and elimination of *Peptostreptococcus anaerobius. Antagonist activity of the periodontopathogens S. salivarius, Veillonella parvula* decreased. In subject 2 during the experiment the periodontopathogen antagonist *S. salivarius* became slightly active, but in parallel the pathogen *S. sanguis* (periodontium) and *Prevotella melaninogenica* (back of tongue) became active. In subject 3 during the experiment the activation of the main periodontopathogens (*P. melaninogenica, Fusobacterium sp., Actinomyces naeslundii, S. sanquis*) is observed against the background of a decrease in the number of natural antagonists of periodontopathogenic flora (*S. salivarius Veillonella parvula*). In subject 4 the activation of fusobacteria and actinomycetes was observed during the experiment (*Fusobacterium sp., A. naeslundii, A. israelii, A.viscosus*). The second group showed similar trends. Microorganisms of the species *P. melaninogenica*, which were completely absent in the baseline cultures of all participants in the experiment, were isolated at the end of the experiment in one subject from the periodontal sulcus ($10^4$ CFU). A continuing trend towards activation of actinomycetes that were absent from the baseline plots should be noted. Two members of the group had *A. naeslundii* ($10^5$ CFU) and *A. israelii* ($10^4$ CFU) in their dental cultures.

There was a slight trend towards a lower concentration of immunoglobulin A in the subjects, possibly due to immune system suppression. The concentration of immunoglobulin G, on the other hand, increased in the group as a whole, apparently reflecting signs of inflammation in the context of qualitative changes in oral microbiocinosis, in particular an increase in the colonization of the oral cavity with periodontopathogenic flora.

The secretory immunoglobulin A, which describes the state of local humoral immunity, showed a unidirectional downward trend in this group, which in turn may contribute to further disruption of oral microbiocoenosis (*Table 4*).

*Anti-orthostatic hypokinesia*

Immunological and microbiological indices reflecting the effect of 60- and 120-day anti-orthostatic hypokinesia on periodontal tissues were studied in the subjects reflected the condition of 60- and 120-day anti-orthostatic hypokinesia (ANOH). During the experiment an increase in the number of periodontopathogenic bacteria *Prevotella melaninogenica, Actinomices naeslundii* and *Fusobacterium nucleatum* against the background of disappearance of bacterial groups (*Streptococcus salivarius* and *Veillonella*

*Table 5*

***Immunoglobulin content (mg/ml) in the oral fluid of subjects with 120 days hypokinesia***

| Immunogloboulins | Terms of experiment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Before | 7 days | 30 days | 60 days | 90 days | 120 days | 7 days after | 30 days after |
| S-IgA | 0,23 ± 0,16 | 0,37 ± 0,12 | 0,69 ± 0,16 | 0,10 * ± 0,05 | 0,37 ± 0,12 | 0,29 ± 0,06 | 0,35 ± 0,05 | 0,23 ± 0,05 |
| IgA | 0,01 ± 0,01 | 0,03 ± 0,01 | 0,07 * ± 0,03 | 0,04* ± 0,03 | 0,05 * ± 0,01 | 0,03 * ± 0,04 | 0,02 * ± 0,03 | 0,02 ± 0,03 |
| IgG | 0,02 ± 0,02 | 0,02 ± 0,02 | 0,09 ± 0,05 | 0,09 * ± 0,05 | 0,22 * ± 0,04 | 0,10 * ± 0,03 | 0,10 * ± 0,03 | 0,09 * ± 0,01 |

* – significant difference compared to data before the experiment, p * 0.05.

*Table 6*

***Species and quantitative composition of the gingival sulcus in subjects undergoing 60-day hypokinesia***

| Microflora | Terms of experiment | | | | | |
|---|---|---|---|---|---|---|
| | Background | 7 days | 30 days | 60 days | + 7 days | + 30 days |
| | ***Subject A*** | | | | | |
| *Streptococcus sanguis* | 6 | 6 | 8 | 7 | 6 | 6 |
| *S.salivarius* | 4 | 4 | - | - | - | 5 |
| *Peptostr. anaerobius* | 5 | 5 | 6 | 7 | 5 | 5 |
| *Actinomyces naeslundii* | 4 | 4 | 6 | 6 | 4 | 4 |
| *Fusobacterium sp.* | 4 | 4 | 6 | 5 | 4 | - |
| *Prevotella. melaninogenica* | - | 3 | 4 | 5 | - | - |
| | ***Subject B*** | | | | | |
| *Streptococcus sanguis* | 6 | 6 | 8 | 8 | 8 | 5 |
| *Peptostr. Anaerobius* | 5 | 4 | 5 | 5 | 5 | 5 |
| *Prevotella.melaninogenica* | - | - | 7 | 6 | - | - |
| *Veillonella* | 4 | - | - | - | - | 4 |
| *Peptostr. intermedius* | 5 | 4 | 5 | 5 | 5 | 5 |
| | ***Subject C*** | | | | | |
| *Streptococcus sanguis* | 7 | 6 | 6 | 7 | 6 | 4 |
| *Peptostr. anaerobius* | 6 | 5 | 5 | 6 | 5 | 3 |
| *Fusobacterium sp.* | 4 | 4 | 4 | 5 | 5 | - |
| *Peptostr. intermedius* | 4 | 4 | - | 5 | - | 3 |
| | ***Subject D*** | | | | | |
| *Streptococcus sanguis* | 5 | 7 | 6 | 6 | 6 | 6 |
| *Peptostr. anaerobius* | 5 | 7 | 5 | 6 | 5 | 4 |
| *Actinomyces.naeslundii* | 4 | 6 | 4 | - | 4 | 4 |
| *Fusobacterium sp.* | 4 | 6 | 5 | - | - | - |
| *Prevotella.melaninogenica* | - | 6 | 6 | - | - | - |
| *Veillonella* | 4 | - | - | 4 | 4 | 4 |

*spp.*) which constitute normal oral microbiocenosis was shown. The content of immunoglobulins before the experiment was normal. During the experiment the number of immunoglobulins increased significantly, which seems to indicate sensitization of tissues by oral antigens, mainly toxins of microorganisms (*Tables 5* and *6*).

Thus, the studies give reason to consider the extreme influences we studied, defined mainly as a specifically altered environment, as a set of factors triggering the development of pathological changes in the dentoalveolar system. These changes can eventually transform into manifest forms of disease. 60- and 120 – day ANOH leads to unidirectional changes on the part of the microbiocenosis and immunity, which is expressed in the appearance and progressive quantitative increase in periodontopathogenic bacteria and an increase in the content of immunoglobulins in the oral fluid.

*Microflora and local periodontal immunity in astronauts*

As a result of long-term orbital flight, the composition of oral microbiocenosis was significantly disturbed [5, 6], both qualitatively and quantitatively, which was expressed in the appearance of a number of periodontopathogenic species, in particular *Actinomices naeslundii, Prevotella melaninogenica, Fusobacterium nucleatum* and a significant increase in the number of bacteria species that can support the inflammatory process (*Table 7*). These changes were mostly pronounced on the first day after completion of the flight and gradually disappeared by the 14 day. As a result of spaceflight factors, including landing, there was an increase of immunoglobulins in the oral fluid on the first day: S-IgA from 0.07 ± 0.04 to 0.27 ± 0.09 (mg/ml); IgA from 0.06 ± 0.01 to 0,07 ± 0.04 (mg/ml); IgG from 0.1 ± 0.02 to 0.22 ± 0.04 (mg/ml) and especially the seventh day: S-IgA, 0.35 ± 0.11 (mg/ml); IgA, 0.19 ± 0.04 (mg/ml); IgG, 0.37 ± 0.10 (mg/ml). Decrease of

*Table 7*

***Gingival sulcus microflora in astronauts (lg CFU neck of the 6th lower tooth left and right)***

| Astronauts | Terms | Microorganisms | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Streptococcus sanguis* | *S.salivarius* | *Veillonella spp.* | *Prevotella melaninogenica* | *P. ogalis* | *P.anaerobius* | *Actinomyces naeslundii* | *A. israelli* | *Fusobacterium nucleatum* |
| 1 | -15 days. | 7 | 6 | 5 | - | 5 | 5 | - | - | - |
| | +1 day. | 7 | 6 | - | 4 | 5 | 5 | 4 | 5 | - |
| | +7 days | 7 | 7 | - | - | - | 6 | - | 5 | - |
| | +14 days | 6 | 7 | - | - | 5 | 6 | - | 6 | - |
| 2 | -15 days. | 6 | 6 | - | - | 5 | 5 | - | - | - |
| | +1 day. | 7 | 6 | - | - | 6 | 6 | 5 | - | 5 |
| | +7 days | 6 | 5 | - | - | 6 | 6 | - | - | 5 |
| | +14 days | 7 | 6 | 5 | - | - | 7 | - | - | 5 |
| 3 | -15 days. | 7 | 6 | 5 | - | 5 | 6 | - | - | - |
| | +1 day. | 7 | 6 | - | 6 | 5 | 6 | 5 | 5 | 5 |
| | +7 days | 7 | 6 | 4 | - | 5 | 7 | - | 5 | - |
| | +14 days | 7 | 7 | - | - | 5 | 7 | - | 5 | - |
| 4 | -15 days. | 6 | 6 | 5 | - | 5 | 5 | - | - | - |
| | +1 day. | 6 | 5 | - | - | 5 | 6 | 5 | - | 6 |
| | +7 days | 7 | 5 | - | 5 | - | 6 | - | - | - |
| | +14 days | 6 | 5 | 6 | - | - | 6 | - | - | - |
| 5 | -15 days. | 6 | 7 | - | - | - | 6 | - | - | 5 |
| | +1 day. | 6 | - | - | - | 5 | 7 | - | - | 6 |
| | +7 days | 7 | 7 | - | - | 6 | 6 | - | - | 5 |
| | +14 days | 6 | 7 | 5 | - | 5 | 6 | - | - | 5 |

* –15 days – pre-flight; +1, +7, +14 days – post-flight.

immunoglobulin level occurred on 14 day S-IgA – 0,13 ± 0,03 (mg/ml); IgA – 0,08 ± 0,01 (mg/ml); IgG – 0,06 ± 0,04 (mg/ml) with normalization of oral microbiocenosis.

## Conclusion

Thus, the experience of the conducted researches testifies to development of infringements of colonization resistance of periodontium practically in all cases of use by the person of the artificially changed environment of an inhabitancy. At the same time, determinants for the development of this syndrome were both specific factors, i.e. factors of altered environment, and nonspecific, presumably, stressinduced factors.

These circumstances make it necessary to develop a system of non-biocidal preventive measures, mainly using probiotics, in particular autoprobiotics.

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