

## THE DENSITY OF MICROFLORA IN THE ORAL AND PHARYNGEAL CAVITIES

I. PAVLÍK, R. ŠTUKOVSKÝ, M. RUSINKO

Research Institute of Human Bioclimatology and Institute of Microbiology of the Comenius University Medical Faculty, Bratislava, Czechoslovakia

It has been found that the bioelectric potential in the oral and pharyngeal cavities is quite regularly distributed (10, 11) so that an electrostatic filter is formed the positive pole of which is situated on the tonsils and on the mesopharynx rear wall, while the negative pole is the tongue surface (Fig. 1). Hence, electrically charged particles must be influenced by this electrostatic field, the electromotive force of this field being considerable; due to the minute distance between the tonsils and the tongue surface it nearly corresponds to a gradient of  $2 \times 10^5 \text{V/m}$  which is capable of bringing a microbe in a second or even in a fraction of a second from the tongue lateral surface to the tonsils. In this context the findings of *Mycobacteria tb* in excised tonsils of clinically nontuberculous children are interesting (3, 6, 7). As all microbes — not only *M. tb* — possess a negative electrokinetic potential in aqueous solutions with a pH value above 5 (2), their isoelectric point being at about 2–4, they must be concentrated on the positive pole of the natural electrostatic filter, i. e., on the tonsils and on the pharynx wall. If this were so, it would mean that the bioelectric potential of the mucous membranes in the oral and pharyngeal cavities has passed through an adaptive process during evolution so that it is now functioning in synergism with the immunocompetent tissue of the Waldeyer-Pirogov lympho-epithelial ring where antibodies are normally produced (1, 5, 13, 14, 15, 18). A successful electrotherapeutic method used in tonsillopharyngitis can be taken as confirmation of this idea (12).

In order to prove this logical presumption it was necessary to assess quantitatively the density of microflora on different spots of the oral and pharyngeal cavities. This was carried out by means of the method already described in principle (9).

### MATERIALS AND METHODS

3.5 per cent agar/No. 1 of the Fa Imuna/with 5 per cent of glycerol was sterilized at 120 °C for 20 minutes and 4 ml of the liquid were poured into sterile glass tubes

Table 1. Basic values of microbe density (N/cm<sup>2</sup>) in 15 subjects

| Subject | Sex | Spot      |            |            |           |            |
|---------|-----|-----------|------------|------------|-----------|------------|
|         |     | Tongue    |            | Fossils    |           | Pharynx    |
|         |     | left      | right      | left       | right     |            |
| 1       | w   | 103079.0  | 103079.0   | 156766.0   | 120258.8  | 68719.3    |
| 2       | w   | 86972.9   | 88046.7    | 476740.4   | 76235.5   | 740880.4   |
| 3       | m   | 469224.3  | 510026.4   | 870995.3   | 1932731.6 | 309237.0   |
| 4       | w   | 115963.9  | 62276.9    | 410168.6   | 140659.9  | 38654.6    |
| 5       | m   | 62276.9   | 90194.1    | 146028.6   | 49392.0   | 53687.0    |
| 6       | w   | 499289.0  | 642096.4   | 1175745.0  | 1159639.0 | 588409.4   |
| 7       | w   | 193273.2  | 299573.4   | 4262746.9  | 163208.4  | 3221.2     |
| 8       | m   | 77309.3   | 144954.9   | 677529.8   | 80530.5   | 57981.9    |
| 9       | w   | 278098.6  | 63350.6    | 127775.0   | 123480.1  | 10737.4    |
| 10      | m   | 98784.0   | 964218.3   | 2607040.2  | 1894077.0 | 89120.4    |
| 11      | m   | 321048.2  | 128848.8   | 191125.7   | 46170.8   | 96636.6    |
| 12      | w   | 109521.4  | 192199.4   | 853811.7   | 789198.7  | 322.1      |
| 13      | w   | 809599.8  | 823558.4   | 190051.9   | 424127.2  | 8589.9     |
| 14      | m   | 261992.5  | 356481.6   | 688267.2   | 395136.2  | 23622.3    |
| 15      | m   | 1191851.1 | 10458225.4 | 10157578.2 | 7580602.8 | 11392379.0 |
| Mean    |     | 311885.6  | 995142.0   | 1532824.7  | 998363.2  | 898813.2   |
|         |     | 653513.80 |            | 1265593.95 |           |            |

10 cm in length and 0.77 cm in diameter. Both ends of the glass tube were closed with sterile rubber plugs. The glass tubes with the agar columns were stored either at 4°C or at room temperature when used next day.

In order to avoid or at least to minimize the influence of the mechanical forces (saliva flux, tongue movements, sweeping away by swallowing) the subjects were examined immediately after the night's sleep at 7.<sup>30</sup> a. m. before having drunk, eaten, smoked, or cleaned their teeth.

Table 2. Tenfold inoculation of the same concentration from one subject

| Items of the blood agar plates | Tongue left number of colonies | Tongue right number of colonies |
|--------------------------------|--------------------------------|---------------------------------|
| 1                              | 251                            | 116                             |
| 2                              | 233                            | 96                              |
| 3                              | 302                            | 132                             |
| 4                              | 273                            | 128                             |
| 5                              | 283                            | 108                             |
| 6                              | 255                            | 130                             |
| 7                              | 220                            | 107                             |
| 8                              | 274                            | 120                             |
| 9                              | 246                            | 163                             |
| 10                             | 227                            | 89                              |
| Mean                           | 256.4                          | 118.9                           |
| SD                             | ±26.35                         | ±21.11                          |
| SE = $\sqrt{\frac{SD}{10}}$    | ± 8.33                         | ± 6.68                          |

The processing was as follows: the glass tube was opened and the agar column pushed with a piston till it protruded about 0.5 cm. The free end of the agar column was cut off with a sterile scalpel close to the tube orifice so that a smooth circular agar area 0.77 cm in diameter was gained. Then the agar column was pushed another 0.1 cm and the free end was pressed for 3 seconds with an approximately standard mild force against the surface of the tongue about 2 cm above the tongue tip, symmetrically on both

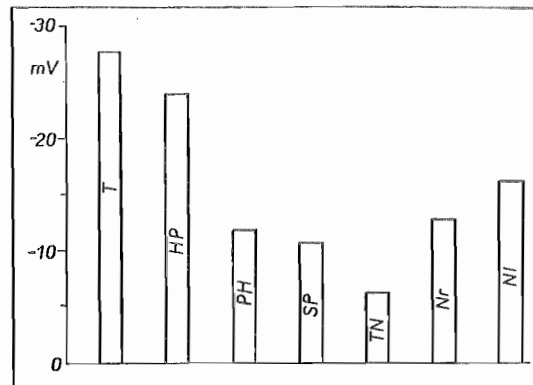


Fig. 1. A survey of average biopotential values on different spots in 120 subjects. Used symbols: T = tongue, TN = tonsil, PH = pharynx; HP = hard palate, SP = soft palate, N = nose, r = right side, l = left side.

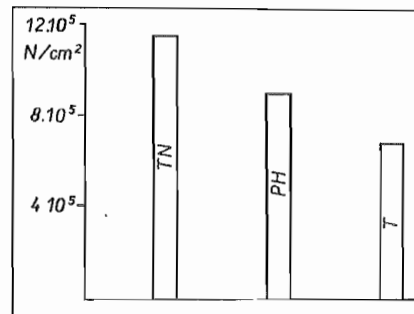


Fig. 2. The sequence of arithmetic means of measured density of microflora on the oral and pharyngeal mucosa in 15 subjects. The sequence is identical with that found previously in other 15 subjects.

sides of the median line, then against the tonsils and finally against the pharynx rear wall. For each spot, a new glass tube was used for the material thus gained. The agar column was then pushed with the piston and the free end with the microbial print, about 0.5 cm in length, was cut off under sterile conditions and dropped into a 20 ml bottle containing 10 ml of sterile phosphate buffer pH = 7.2. The samples were immediately processed in the laboratory in a shaking device for 20 min, then an amount 0.5 ml of the liquid from each bottle was diluted to three different concentrations 1 : 10, 1 : 100, and 1 : 1000. From each concentration (including that of 1 : 1 or undiluted liquid) twice 0.1 ml were inoculated on two blood agar plates and incubated for 18--20 hours at 37 °C under aerobic conditions. The number of microbial colonies of the

optimal dilution was counted and reduced for 1 cm<sup>2</sup> of surface of the respective spot in the oral or pharyngeal cavity by multiplying with the dilution ratio (10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup>, or 10<sup>5</sup>) and with the factor 2.14748 (= 1 : π x 0.385<sup>2</sup>). The total number of adult subjects examined was 15 (8 females and 7 males). In several cases the tonsils and the tongue were coloured with edible dye and prints were made with the agar columns on white



Fig. 3. A nest of several hundred microbes in a stained saliva smear. Magnification 1000 x. Staining; Pappenheim.

Table 3. Main parameters of the measurements on 5 spots in 15 subjects

| Sampling spot | in logarithms |          | retransformed            |       |
|---------------|---------------|----------|--------------------------|-------|
|               | $\bar{X}$     | S. D.    | mean                     | S. D. |
| Tongue left   | 5.31622       | ±0.39614 | 20.712 × 10 <sup>4</sup> | 2.49  |
| Tongue right  | 5.43515       | ±0.59372 | 27.237 × 10 <sup>4</sup> | 3.92  |
| Tonsil left   | 5.79870       | ±0.56554 | 62.908 × 10 <sup>4</sup> | 3.68  |
| Tonsil right  | 5.48713       | ±0.66847 | 30.700 × 10 <sup>4</sup> | 4.66  |
| Pharynx       | 4.75953       | ±1.07519 | 5.748 × 10 <sup>4</sup>  | 11.89 |

t  
5 %  $M\sqrt{\frac{\sigma}{n}} = t, m$  : Tongue left ± 0.21887  
Tongue right ± 0.32803  
Tonsil left ± 0.31246  
Tonsil right ± 0.36933  
Pharynx ± 0.59405

Table 4. T-test for differences of mean log values in 15 subjects

| Sampling spot | Tongue |          | Tonsils  |          | Pharynx  |
|---------------|--------|----------|----------|----------|----------|
|               | left   | right    | left     | right    |          |
| Tongue left   | —      | -0.11893 | -0.48248 | -0.17091 | +0.55668 |
| Tongue right  | —      | ±0.42481 | ±0.58846 | ±0.53942 | ±1.01156 |
| Tonsil left   | -1.08  | —        | -0.36355 | -0.05198 | +0.67561 |
| Tonsil right  | n. s.  | —        | ±0.41572 | ±0.33221 | ±0.96056 |
| Pharynx       | -3.18  | -3.39    | —        | ±0.31157 | +1.03917 |
|               | <0.01  | <0.01    | —        | ±0.47865 | ±1.05067 |
|               | -1.23  | -0.61    | +2.52    | —        | +0.72760 |
|               | n. s.  | n. s.    | <0.05    | —        | ±1.06663 |
|               | +2.13  | +2.72    | +3.83    | +2.64    | —        |
|               | n. s.  | <0.02    | <0.01    | <0.02    | —        |

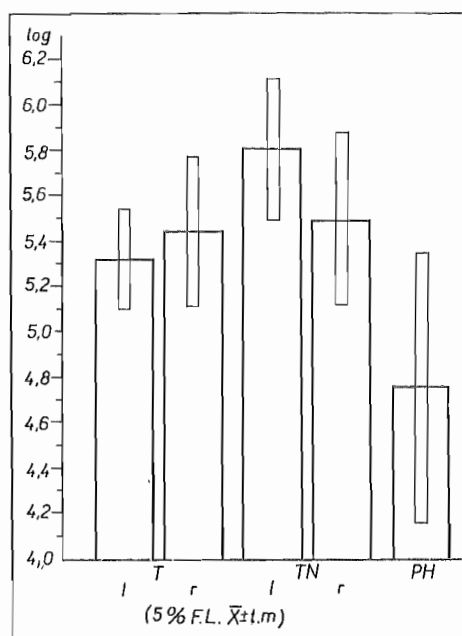


Fig. 4. Arithmetic means of the logarithmically transformed values, i.e., geometric means in fact, with 95 per cent fiducial limits. (F. I. = confidence limit).

drawing paper in order to be sure that the whole surface of the circular area came into contact with the surface of the mucous mebrane of the spot under investigation. The prints on the paper were perfect.

#### RESULTS

A survey of the results is given in Tab. 1 and Fig. 2. The source of the very high variability of the values is in the sampling conditions as the inoculation of one sample on 10 agar plates had only a small dispersion (Tab. 2). It is uncer-

tain how much contamination of the side wall of the agar column and the appearance of microbes in small clusters on the surface of the mucous membranes respectively contribute to the high variability. (Fig. 3).

Having in mind this high variability, a logarithmic transformation of the individual values was performed before the statistical evaluation (17). The mean logarithmic values and their standard deviations as well as the retransformed

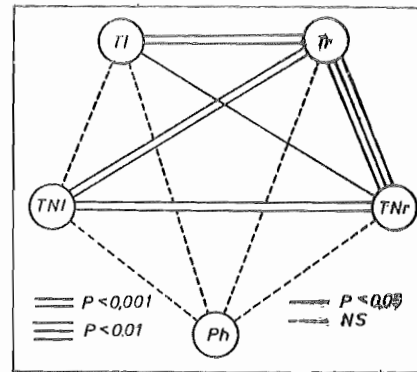
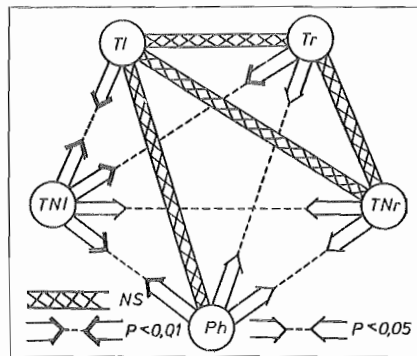


Fig. 5. Significance of differences between the means of log values. T = tongue, TN = tonsil, PH = pharynx, l = left side, r = right side.

Fig. 6. Correlational relations between the microflora density on the spots examined.

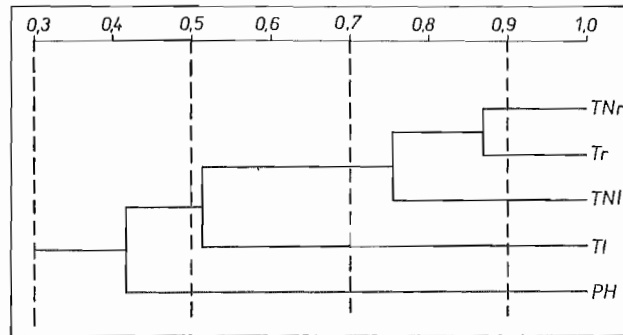


Fig. 7. A dendrogram showing a cluster formation on the basis of correlational proximity. On the horizontal axis is the value of the correlation coefficient.

values are presented in Tab. 3 and Fig. 4. It may be seen that the sequence changed after the transformation: the lowest number corresponds now to the pharynx. The significance of the differences between the mean values was then calculated, with results as summarized in Tab. 4 and Fig. 5.

The correlation between the density of microflora on single spots is presented in Tab. 5 and Fig. 6. An analysis by the weighed variable-group method led to the dendrogram showing a cluster formation on the basis of the correlational proximity.

DISCUSSION

The pattern of the microflora inhabiting the mouth and the pharyngeal cavity may be very diverse: the anaerobes prevail in the folds where the oxygen tension is less than 1 per cent of the barometric pressure (4). In the parts under investigation where the oxygen tension reaches 12.4—16.4 per cent, the most frequent inhabitants are aerobic microbes such as *Streptococcus mitis*, *Neisseria pharyngis*, nonpathogenic Staphylococci, Corynebacteria, Haemophili etc. (16). Thus, the cultivation was limited to aerobic conditions.

Table 5. Correlations between the log values

| Sampling spot | Tongue |        | Tonsils |       | Pharynx |
|---------------|--------|--------|---------|-------|---------|
|               | left   | right  | left    | right |         |
| Tongue left   | —      | 0.699  | 0.291   | 0.591 | 0.340   |
| Tongue right  | <0.01  | —      | 0.744   | 0.868 | 0.459   |
| Tonsil left   | n. s.  | <0.01  | —       | 0.711 | 0.306   |
| Tonsil right  | <0.05  | <0.001 | <0.01   | —     | 0.324   |
| Pharynx       | n. s.  | n. s.  | n. s.   | n. s. | —       |

$$r_{5\%} = 0.514, r_{1\%} = 0.641, r_{0.1\%} = 0.760$$

Now the question arises how reliable are results which are subject to such a high interindividual variability. There is a fundamental consideration based on obtaining repeatedly the same sequence of arithmetic means of the microbial density on the tongue, the tonsils, and in the pharynx. In two different labora-

Table 6. The influence of teeth cleaning and eating breakfast upon the microflora density (N. cm<sup>-2</sup>)

| Sampling spot | Before breakfast |       |        | After breakfast |      |      | Difference of the means |
|---------------|------------------|-------|--------|-----------------|------|------|-------------------------|
|               | M                | W     | mean   | M               | W    | mean |                         |
| Tongue left   | 5220             | 27510 | 16365  | 1250            | 6564 | 3907 | — 12458                 |
| Tongue right  | 4515             | 24656 | 14585  | 2850            | 1164 | 2007 | — 12578                 |
| Tonsil left   | 198500           | 29907 | 114203 | 1610            | 2557 | 2083 | —112120                 |
| Tonsil right  | 2630             | 13333 | 7981   | 820             | 1878 | 1349 | — 6632                  |
| Pharynx       | 40               | 55477 | 27758  | 635             | 2146 | 1391 | — 26367                 |
| Mean          | 42181            | 30176 | 36178  | 1433            | 2862 | 2147 | — 34031                 |

tories and with the same number of different subjects the same sequence was obtained (10, 11) corresponding exactly to the distribution of the bioelectric potential. When the expectance of a random correspondence is  $1 \times \frac{1}{3!} = \frac{1}{6}$ , then the same result for a second independent experiment has a probability of  $\left(\frac{1}{3!}\right)^2 = \frac{1}{36} = 0.0278$  which means that with a probability as high as 97.22 per cent, the electrostatic filter determines the pattern of the microflora density

on the mucous membrane in the oral and pharyngeal cavities under the conditions described.

The arithmetic mean of the logarithmic values of the pharynx, however, is the lowest, this being due to the widest scatter of the single values. The swallowing act, which cannot be excluded, is evidently the cause. In two subjects, one man and one woman, the microflora density was examined first under usual basic conditions and then after teeth cleaning and breakfasting was examined (Tab. 6). The differences are striking.

A more detailed analysis of the material shows that there are differences between the sampling spots. Seven out of ten t-tests are significant proving the inhomogeneity in the microflora density. The diagram (Fig. 5) shows that the maximal (logarithmic) and minimal (logarithmic) values (i.e., left tonsil and pharynx, respectively) differ significantly from each other and simultaneously from the remaining three values (left side of the tongue, right side of the tongue, and the right tonsil) which do not differ significantly among themselves.

But Table 5 and Fig. 6 demonstrate that there is also an interdependence of the values, higher densities on one spot tending to be associated with higher densities on other spots in the same person. All correlation coefficients are positive and five of them are highly significant. The interpretation of this finding leads to the idea that the pharynx values are practically independent, being disturbed probably by swallowing and on the other hand, that there is a strong correlation between the tongue and the tonsils. It may be suspected that there exists an asymmetry in the density of the microbes on both sides. Unfortunately, the biopotential in the oral and pharyngeal cavities has not been measured with respect to laterality, though, in the nasal cavity, a significant asymmetry of the biopotential has been found (8, 10, 11).

A dendrogram (Fig. 7) summarizes these conditions in a survey showing the closest relation between the right tonsil and the right side of the upper tongue surface forming together a dextralateral nucleus accompanied by the left tonsil. The isolated position of the pharynx is again conspicuous.

According to these findings, there are persons with a generally high or low density of oral microflora, the densities in the oral cavity and on the tonsils being more or less interdependent whereas the density on the pharynx rear wall is independent.

#### S U M M A R Y

A method for quantitative assessment of the microbial density on the mucous membranes in the oral and pharyngeal cavities is described. The results of the assessment are in agreement with the theoretical assumption based on the existence of a natural electrostatic filter. The highest density of microbes is on the tonsils where the positive pole of the filter is situated, so that a synergism between the defensive immunological role of the tonsils and the bioelectric potential pattern is obvious. The microbial densities in the oral cavity are more or less interdependent whereas the findings on the pharynx rear wall are isolated being probably perturbed by repeated swallowing.



#### R É S U M É

I. Pavlík, R. Štukovský, M. Rusínko: **La densité de la flore microbienne dans la cavité buccale et laryngienne**

Les auteurs décrivent une méthode de la détermination quantitative de la densité des microbes sur les membranes muqueuses de la cavité buccale et laryngée. Les résultats de la détermination s'accordent avec la supposition théorique basée sur l'existence d'un filtre électrostatique naturel. La densité la plus haute des microbes se trouve sur les tonsilles ou se trouve le pôle positif du filtre de sorte que la synergie entre le rôle immunologique défensif des tonsilles et la répartition du potentiel bioélectrique paraît évidente. Les densités des microbes dans la cavité buccale sont réciproquement dépendantes tandis que les constatations sur la paroi postérieure du larynx sont isolées étant vraisemblablement dérangées par la déglutition répétée.

#### Z U S A M M E N F A S S U N G

I. Pavlík, R. Štukovský, M. Rusínko: **Die Dichte der mikrobiellen Flora in den Mund- und Rachenhöhle**

Es wird eine Methode der quantitativen Bestimmung der Mikrobendichte an den Schleimhäuten der Mund- und Rachenhöhle beschrieben. Die Ergebnisse der Bestimmung in Übereinstimmung mit der auf die Existenz des natürlichen elektrostatischen Filters gestützten theoretischen Voraussetzung. Die höchste Dichte von Mikroben befindet sich an den Mandeln, wo der positive Pol des Filters ist, so dass der Synergismus zwischen der defensiven immunologischen Aufgabe der Mandeln und der Einteilung des bioelektrischen Potentials offenbar zu sein scheint. Die Dichten von Mikroben in der Mundhöhle sind mehr oder weniger voneinander abhängig, während Befunde an der Hinterwand der Rachenhöhle isoliert sind, denn sie sind wahrscheinlich durch wiederholtes Schlucken gestört.

#### R E S U M E N

I. Pavlík, R. Štukovský, M. Rusínko: **La densidad de la flora microbiana en la cavidad bucal y faringea**

Se describe el método de la determinación cuantitativa de la densidad de los microbios sobre las mucosas de la cavidad bucal y faringea. Los resultados de la determinación son conformes con la suposición teórica apoyada sobre la existencia del filtro electrostático natural. La más alta densidad de los microbios se halla sobre las amígdalas, donde se encuentra el polo positivo del filtro, así que el sinergismo entre la tarea inmunológica defensiva y la división del potencial bioeléctrico parece ser evidente. Las densidades de los microbios en la cavidad bucal se muestran más o menos mutuamente dependientes, mientras que los microbios en la pared posterior de la faringe se hallan más aislados, ya que son estos perturbados probablemente a consecuencia de los tragos repetidos.

#### R E F E R E N C E S

1. Berendes, J., Liuk, R., Zöllner, F.: Hals-Nasen-Ohren-Heilkunde, Vol. II., part 1., Stuttgart, G. Thieme, 1963. — 2. Bier, M.: Electrophoresis. Theory, methods and applications. New York, Acad. Press Inc. 1959. — 3. Dornetzhuber, V., Grigelová, R., Sýkora, F.; Zbl. allg. Path., 112, 1969, p. 12—17. — 4. Eskow, R. N., Loeschke, W. J.:

- Archs oral Biol., 16, 1971, p. 1127—1128. — 5. **Gitlin, D., Sasaki, T.**: Science, 164, 1969, p. 1532—1534. — 6. **Grigelová, R., Markovič, J., Dornetzhuber, V., Sýkora, F.**: Beitr. Klin. Tuberk., 130, 1965, p. 296—300. — 7. **Grigelová, R.**: Z. Tuberk., 127, 1967, p. 77. — 8. **Kvasov, D. G., Marievskaja A. P.**: Fiziol. Zhurn. 45, 1959, p. 959. — 9. **Pavlík, I.**: Čs. Otolaryngol., 13, 1964, p. 204. — 10. **Pavlík, I.**: HNO, 15, 1967, p. 167. — 11. **Pavlík, I.**: Int. J. Biometeor., 11, 1967, p. 175. — 12. **Pavlík, I., Hnátek, J.**: Z. Laryngol. Rhinol. 52, 1973, p. 225. — 13. **Piffkó, P., Kóteles, G. J., Antoni, F.**: Otorhinolaryngol. 34, 1972, p. 10. — 14. **Přecechtěl, A.**: The Streptococcal Angina, Thomayerova sbírka 370, 1957, Praha. SZdN. — 15. **Přecechtěl, A.**: The Tonsils, In: Encyclopaedia of the practising Physician, Part 11, 1957, p. 273, Praha SZdN. — 16. **Přecechtěl, F.**: Medical Microbiology for Stomatologists, Praha, SPN, 1964. — 17. **Snedecor, G. W.**: Statistical Methods Applied to Experiments in Agriculture and Biology, 5th ed., Iowa, The Iowa State College Press, Ames, 1957. — 18. **Vianna, N. Y., Greenwald, P., Davies, J. N. P.**: Lancet I, 1971, p. 431.

Received November 21, 1974

I. Pavlík, Research Institute of Human Bioclimatology,  
800 00 Bratislava, Mickiewiczova 13, Czechoslovakia

Winter, K.: **Soziologie für Mediziner**, Verlag Volk und Gesundheit, Berlin 1973, pp. 193, Figs. 3.

From the beginning of the sixties, there has been a revival of Marxist sociology in the socialist countries. Thus there were conditions for the use of its concepts, theories and methods in medicine, too. In the GDR, for instance, the tuition of medical sociology was quite naturally included in the curriculum of social hygiene (social medicine) and lectures have been given on this subject at Humboldt University in Berlin since 1964 and at all medical faculties from 1967. The book under review was written with this pedagogical process in view.

This pleasantly slim volume was written with the assistance of 4 sociologists, 4 medical doctors and 2 psychologists. Under the editorship of Prof. K. Winter, a leading representative of social hygiene in the GDR, this team was able to compile a comprehensive and very useful handbook from the point of view of medical sociology for medical students. The book opens with a chapter on the relationship between medicine and sociology, continues with

aspect of sociology in the different phases of the life cycle of man and ends with problems of sociological methods of research. The authors are experienced teachers and it is therefore not surprising that the handbook is written competently, objectively, critically and without waste of words.

In connection with the classification, character and relationships of medical sociology to other sciences, a dispute sometimes arises as to whether it is a medical or a socio-scientific discipline. The authors believe that a dispute at this level is pointless. It is true that medical sociology arose as a branch of sociology and belongs to it by its nature, but in fact, it is one of the branches developing on the border of the so far strictly separated scientific disciplines which, under the integrating formative influence of social medicine, deal with the complex problems of human (and thus social) aspects of health and disease. If the results of their work are to be used clinically, it will evidently be necessary in the future for them to form an institutional unit integrated in the framework of social medicine.

Č. Müller