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## TABLE OF CONTENTS

11, 1, 1969

Quigley, Jr. L. F., Cobb C. M.: Clinical and Subjective Evaluation of Speech . . . . .	1
Šurina I., Jágr J.: Action Potentials of Levator and Tensor Muscles in Patients with Cleft Palate . . . . .	21
Marino A.: Reconstructive Plastic Surgery in Extensive Perforations of the Nasal Septum . . . . .	31
Alexandrov N. M.: Resection of the Uper Jaw with Elements of a Primary Plastic . . . . .	38
Klen R., Heger J.: A Contribution to the Degradation of Skin and Cornea Preserved Hemibiotically in the Temperature Near 0°C (Brief Communication) . . . . .	47
Demichev N. P.: Assessing the Biological Activity of Conserved Tendon by Tissue Culture . . . . .	56
Orlov G. A.: Absorbable Synthetic Material Used in Plastic Surgery . . . . .	67
Kazanceva N. D.: Growth Characteristic of Skin Thickness in Children and its Significance in Free Skin Grafts . . . . .	71
Šmahel J.: The Problem of Revascularization of Free Skin Autografts . . . . .	78

11, 2, 1969

Jirsáková A., Holuša R.: The Induction of Cleft Palate by Cortisone in Several Inbred Mice Strains . . . . .	85
Tolarová M.: Microforms of Cleft Lip and or Cleft Palate . . . . .	96
Tolarová M.: Genealogical Analysis of Isolated Cleft Palate . . . . .	108
Jaworska M.: Simian Crease and Congenital Malformations . . . . .	117
Pellant A.: External Auditory Meatus Plasty in Congenital Atresia . . . . .	124
Königová R.: Double Tube Pedicle Flaps in Lower Abdomen . . . . .	130
Troshev K.: Contribution to the Anthropometric Study of the Penis in a Group of Bulgarian Boys from Birth to the Age Seven Years . . . . .	140
Rusakov V. I., Krasulin V. V.: Method of Formation of the Anterior Urethra in Extensive Strictures . . . . .	149
Sedláček J.: Lymphovenous Shunt as Supplementary Treatment of Elephantiasis of Lower Limbs . . . . .	157
Godunov S. F., Keier A. N.: Free Bone Plasty for Lengthening of Short Arm Stumps . . . . .	163
 Annotations	
Berndorfer A.: Basic Principles of the Veau Operation Method . . . . .	175
Boris Vasilyevich Parin . . . . .	178

11, 3, 1969

Samohýl J., Šťastná J.: The Main Types of Granulation in the Electron-Microscopic Picture . . . . .	181
Beringer U. V., Darkshevich U. N.: Dynamics of Morphological Changes after Homografts of Tendons Preserved in Anticytolytic Solutions . . . . .	187
Pavlova M. N., Vyalcev V. V.: Autoplasty and Homoplasty of Extensive Defects of the Vault . . . . .	197
Pap G. S.: The Gillies Fan Flap. Restoration of Substances for Partial Loss of Uooer Lip (Case Report) . . . . .	210
Šurina I., Král A.: Practical Method of Speech Evaluation in Adult Patients with Cleft Lip and Cleft Palate . . . . .	215
Becker R.: Pedagogic Problems in the Rehabilitation of Patients with Cleft . . . . .	221
Ionescu A., Petrovici V.: Complications of Burns . . . . .	228
Königová R.: Some Notes about Treatment of Burns in Great Britain . . . . .	242
Savickij G. A.: Surgical Correction of Sex in Male Pseudohermaphroditism to Female Type . . . . .	247

11, 4, 1969

Hajniš K., Farkaš L. G.: Anthropological Record for Congenital Developmental Defects of the Face (Especially Clefts) . . . . .	261
Jaworska M., Michałowicz R., Stolarska A., Wiszczor-Adamczyk B.: A Case of Structural Chromosomal Anomaly in D (13—15) Group . . . . .	268
Krutchinskyi G. V.: Method of Repair of Upper Lip Defect with a Wedge-Shaped Flap Taken From the Lower Lip . . . . .	276
Kozin I. A.: Surgical Treatment of Deformations of the Uuper Lip and Nose After Plasty of the Lip in Adults with Unilateral Cleft Lip . . . . .	283
Bethmann W., Dyrna J.: On the Problem of Favourable Age and the Most Favourable Method for Palatinal Surgery in Bilateral Total Clefts . . . . .	293
Čečuk L., Prpić I.: Repair After Vulvectomy for Giant Condylomata Acuminata . . . . .	296
Kipikaša A.: Possibilities of the Reconstruction of the Thumb Injure-Loss-Accident . . . . .	301
Degtyareva S. D.: Some Methods of Plastic Reconstruction of Tendons of the Finger Flexors . . . . .	312
Matev I., Stoycheva Y.: Lesions of Finger Aponeurosis at the Level of the Distal Interphalangeal Joint . . . . .	320
Kamayev M. P.: Experiences with the Surgical Treatment of Elephantiasis . . . . .	328

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## CLINICAL AND SUBJECTIVE EVALUATION OF SPEECH

L. F. QUIGLEY, JR., C. M. COBB.

Clinical methods for evaluating velopharyngeal competency which relate directly to speech are urgently needed. Many workers have endeavored to give a precise definition of velopharyngeal competency through the development of objective clinical techniques [1, 2, 3, 20]. Velopharyngeal closure may be estimated directly under static or dynamic conditions through x-rays, photography, or T. V. tape recordings [9]. Closure may be estimated indirectly by measuring nasal airflow and pressure. Currently, a device also exists for evaluating speech nasality through electronic phase shifting techniques, which is currently under investigation by the authors and others [4, 23].

The scope of this investigation was to determine the relationship between airflow during speech and judged nasality.

The two basic tools of subjective speech assessment, word intelligibility and nasality, are limited. Both have inherent disadvantages for clinical evaluations. The procedures for determining either one require the cooperative efforts of a number of trained or untrained speech judges. Modest improvements as might be seen on a month-to-month basis are often not discernible because of the inherent fluctuations of the subjective judging processes. Changes in the composition of the judging group may sometimes cause wide variations in speech assessments so that it is desirable to use the same judges over the course of treatment no matter how prolonged it may be [13, 21, 22].

There is also a basic uncertainty in the subjective judging process itself, particularly with regard to the assessment of nasality. Many times speech defects commonly associated with nasality, such as excessive loss of sibilance [8], may confuse the judges. This is a reasonable result since nasality assessment is not only carried out by the frequency sensing mechanisms of the ear, but by the judges' total remembered experience with speech sounds. It is

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\* ) Presented at the 1965 meeting of the American Cleft Palate Association, New York City, New York. This study was supported in part by The Hood Dairy Foundation and The Medical Foundation.

recognized that nasality may not be the most useful concept for characterizing the defective speech associated with cleft palate patients. However, it is one of the most commonly used criteria by workers in the field of basic speech research. It has been investigated by numerous workers for many years, and a large fund of clinical and laboratory data has been accumulated about this quality of speech (7, 11, 14).

Table 1. Hoyt Reliability Coefficients

1. Maximum Airflow Data			
	Face Mask	Oral Mask	
Expiration	.959	.903	
Inspiration	.923	.947	
2. Airflow During Speech			
Phrase	Face Mask	Oral Mask	Nasal Mask
1	.983	.979	.975
2	.984	.988	.990
3	.989	.978	.940
4	.986	.931	.988
5	.986	.980	.980
6	.978	.971	.995
7	.975	.980	.976
8	.980	.954	.994
9	.945	.957	.986
3. Airflow During Pressure Measurements			
	Hunter	Chase	Vital Capacity
Expiration			
Pressure	.980	.942	.992
Flow	.996	.995	.986
Inspiration			
Pressure	.986	.889	
Flow	.989	.991	

In the current report, both direct and indirect objective techniques have been compared with the patients' subjective judged nasality. The direct techniques are those in which airflow measurements are made during speech. Indirect techniques are those in which measurements of subjective nasality are compared with measurements of simultaneous airflow and pressure under non-speaking conditions.



## PROCEDURES

### Airflow

Airflow was measured with the hot wire anemometer, previously described in the literature (5, 17). The sensing unit consists of a heated wire filament and unheated wire filament, both exposed to the airstream and contained in a "T" shaped probe. A feedback system automatically supplies just enough electric current to the heated filament to maintain its temperature at a fixed ratio to the temperature of the unheated filament. Higher velocities of air



Fig. 1. Hot Wire Anemometer with Nasal and Facial Masks „T“ Probe in Nasal Mask

have a greater cooling effect on the heated filament and automatically call for more current to maintain the temperature ratios. It is this current that indicates the air speed. The small sizes of the sensing elements (approximately .04 inches in length and .0005 inches in diameter) result in negligible airflow interference.

Extremely sensitive and rapid response to minute variations in the velocity of airflow through the metal tube are observable during the breathing cycle.

Three types of masks may be attached to the "T" probe — a nasal mask, a face mask, or an oral mask (Fig. 1). Airflow measurements were made using all three masks for the nine test phrases: sustained (i), (u), and (a), mama, meme, puppy, sixty-six, kitty, Coca Cola. All measurements were taken five times and the results averaged. The Hoyt reliability coefficients for the three masks and the nine test phrases are shown in Table 1. All coefficients are very

high indicating that variation among the five separate readings is very small compared to the variation among subjects. This simply assures one of the reproducibility of the readings.

### 1. Facial

The total airflow from the nasal and oral cavities was measured using the facial mask shown in Fig. 2. This is a commercially available anesthetist's face mask which gives minimum interference with lip movement and minimizes turbulence and back pressure.

Table 2. Analysis of Nasality Errors  
Hoyt Reliability Coefficients for Trained Judges

	15 Subjects Normal	20 Subjects Cleft Palate
Vowel Nasality		
(i)	.74	.77
(u)	.58	.69
(a)	.34	.80
Nasality in Connected Discourse	.71	.90

Readings of facial airflow were made for various tests. In one set of tasks, the patient was instructed to speak the nine test phrases listed above. During the speaking of a test phrase, the airflow varied slightly. The airflow always started at a low level, increased to a plateau, and then fell off as the phrase was terminated. The operator recorded the airflow as closely as possible at the midpoint of the plateau.

Table 3. Description of Subjects According to Age, Sex and Type of Cleft

Sex	No.	Age (mean)	Lip and palate			Palate only		
			Unilat.		Bilat.	Soft & Hd.	Soft	Total
			Left	Rt.				
M	21	11.8 yr.	6	3	6	5	1	21
F	16	11.8 yr.	4	2	1	9		16
	37							37

### 2. Oral

Oral airflows were measured by placing the nasal mask over the oral cavity. The mask is so shaped that nasal airflow is not impeded when it is used in this position. The mask was used to measure airflows during the speaking of the nine test phrases, and to measure oral airflows during maximum expiration and inspiration. Readings were made during the plateau as previously described (Fig. 3).

### 3. Nasal

To measure nasal airflow, a nasal mask was placed over the nasal cavity. The nasal mask was chosen to provide a minimum of lip interference. The effect of the mask upon lip motion will be reported in another publication. The mask is commercially available and has the advantage of a design which mini-



Fig. 2. Facial Mask on Patient

mizes dead airspace, turbulence and back pressures (17). (Fig. 4.) Nasal airflow was measured for the nine test phrases and also under other conditions. Simultaneous pressure measurements were made during measurement of nasal airflow. Three types of pressure instruments were used to provide a range of

Table 4. Normal and Cleft Palate Maximum Flow Measurements

Face Mask								
Normal				Cleft Palate				
	N	Mean Av. Flow	S. D.	N	Mean	S. D.	F Ratio	Com.
Expiration	9	2655.6	394.8	34	2532.4	606.2	0.319	N. S.
Inspiration	8	2820.0	390.6	34	2546.8	683.4	1.131	N. S.
Oral Mask								
Normal				Cleft Palate				
	N	Av. Flow	S. D.	N	Av. Flow	S. D.	F Ratio	Com.
Expiration	8	2705.0	417.6	15	2724.7	256.5	0.018	N. S.
Inspiration	8	2862.5	311.5	15	2674.7	351.7	1.469	N. S.

N. S. Not Significant



pressures: (1) modified wet spirometer (vital capacity manometer) which gave slight resistance, (2) the Chase manometer which was adjusted to give moderate resistance, and (3) the Hunter manometer which was adjusted to give marked resistance. Each pressure measurement was made five different times and the results averaged. The precision of measurement is shown in Table 1.

### Subjective Nasality

The speech of all cleft patients was recorded on an Ampex Series 350 tape recorder using a condenser microphone under standardized conditions. The



Fig. 3a.

nine test phrases used in the airflow measurements [sustained (i), (u), (a), mama, meme, puppy, kitty, sixty-six and Coca Cola] and a paragraph of "Arthur the Young Rat" were recorded by each patient.

These recordings were judged for vowel nasality and nasality in connected discourse for the present correlation studies. Vowel nasality was taken separately for sustained (i), (u), (a). Judging was done by both trained and untrained judges. The three trained judges were all speech workers with Ph. D. degrees. Eight adult untrained judges from the normal population were used.

Nasality was judged by both groups using a seven point scale: 1 — normal, 2 or 3 — slight, 4 to 5 — moderate, and 6 to 7 — severe. The results for a given nasality were obtained by straightforwardly adding the estimates of the three judges.

The value for nasality used was obtained by averaging the three vowel nasalities and the nasality in connected discourse. The averaging was done

separately for both groups of judges and the nasalities produced by each group were finally averaged. In this manner the trained judges were given equal weight with the untrained judges.

The consistency with which the trained judges estimated nasality was examined for the trained judges but not for the untrained judges. Hoyt reliability coefficients for the trained group of judges are shown in Table 2. These data show the trained judges evaluated both the normal and cleft palate patients in a consistent manner. Each of the trained judges made estimates of the different nasalities which agreed with those of his fellows.



Fig. 3a, b. Oral Mask on Patient

### Subjects

A description of the cleft palate patients who were given cephalometric x-rays and nasality analyses is given in Table 3. Thirty-seven cleft palate patients and fourteen normal subjects were studied. An attempt to reduce the number of possible variables was made by restricting the normal subject population to that of siblings of the cleft palate patients. None of the normal patients had any obvious speech defect. All patients, both cleft and normal, were between ages nine and fifteen. The thirty-seven cleft palate patients consisted of twenty-one males and sixteen females. Each group had an average mean age of approximately twelve years. The location of the cleft is indicated in the table. None of the cleft palate patients had had a posterior pharyngeal flap operation.

The distribution of the judged nasality for the cleft palate and normal groups are shown in Fig. 5 and 6. Each figure contains the average and the standard deviation.

Table 5. Normal and Cleft Palate Flow Measurements (ft./min.)

Face Mask								
Normal Subjects				Cleft Palate Patients				
Sound	N	Mean	S. D.	N	Mean	S. D.	F Ratio	Com.
i	14	357.3	118.3	58	325.4	171.0	.425	N. S.
u	14	362.3	118.9	58	346.2	192.9	.086	N. S.
a	14	351.1	126.5	58	336.9	181.8	.074	N. S.
Mama	13	305.2	109.2	56	302.2	143.0	.005	N. S.
Meme	14	351.4	121.0	57	325.8	162.6	.297	N. S.
Puppy	14	339.6	122.7	57	346.6	205.2	.014	N. S.
66	14	395.5	159.4	57	438.6	247.0	.376	N. S.
Kitty	14	331.0	134.1	54	384.3	209.9	.791	N. S.
Coca Cola	4	440.0	88.0	19	430.5	248.6	.005	N. S.

N. S. Not Significant

In addition to the subjects whose nasality was judged and who are described in Table 3, forty-two other cleft palate patients were studied for airflow characteristics. These patients were also between the ages of nine and fifteen. These patients were included to obtain a larger sampling of pressure and airflow data. They were a part of a larger longitudinal study (15). Their attributes are not included in the correlation studies between nasality, pressure and airflow.

## RESULTS

## Flow Measurement of Normal and Cleft Palate Patients

## 1. Maximum Flow Measurements

The maximum airflows which could be generated by normal and cleft palate patients during expiration and inspiration were measured using the face

Table 6. Normal and Cleft Palate Flow Measurements (ft./min.)

Oral Mask								
Sound	Normal Subjects			Cleft Palate Patients				
	N	Mean	S. D.	N	Mean	S. D.	F Ratio	Com.
i	10	439.0	141.9	41	229.5	97.0	29.472	**
u	10	472.0	142.2	41	285.9	145.8	12.706	**
a	10	439.5	155.4	40	284.0	99.5	14.561	**
Mama	10	140.0	48.0	39	162.1	65.7	.951	N. S.
Meme	10	125.0	40.3	40	135.6	74.7	.178	N. S.
Puppy	10	299.5	56.1	40	274.6	135.1	.313	N. S.
66	10	416.5	106.3	40	357.8	186.8	.878	N. S.
Kitty	10	293.5	76.1	40	247.8	94.7	1.930	N. S.
Coca Cola	5	420.0	51.0	25	321.0	106.3	3.872	N. S.

\* Significant at 5% level

\*\* Significant at 1% level

N. S. Not Significant at 5% or 1% levels



## PROCEDURES

### Airflow

Airflow was measured with the hot wire anemometer, previously described in the literature [5, 17]. The sensing unit consists of a heated wire filament and unheated wire filament, both exposed to the airstream and contained in a "T" shaped probe. A feedback system automatically supplies just enough electric current to the heated filament to maintain its temperature at a fixed ratio to the temperature of the unheated filament. Higher velocities of air



Fig. 1. Hot Wire Anemometer with Nasal and Facial Masks „T“ Probe in Nasal Mask

have a greater cooling effect on the heated filament and automatically call for more current to maintain the temperature ratios. It is this current that indicates the air speed. The small sizes of the sensing elements [approximately .04 inches in length and .0005 inches in diameter] result in negligible airflow interference.

Extremely sensitive and rapid response to minute variations in the velocity of airflow through the metal tube are observable during the breathing cycle.

Three types of masks may be attached to the "T" probe — a nasal mask, a face mask, or an oral mask (Fig. 1). Airflow measurements were made using all three masks for the nine test phrases: sustained (i), (u), and (a), mama, meme, puppy, sixty-six, kitty, Coca Cola. All measurements were taken five times and the results averaged. The Hoyt reliability coefficients for the three masks and the nine test phrases are shown in Table 1. All coefficients are very

Table 7. Normal and Cleft Palate Flow Measurements (ft./min.)

Sound	Normal Subjects			Cleft Palate Patients				
	N	Mean	S. D.	N	Mean	S. D.	F Ratio	Com.
u	13	5.8	11.9	61	118.4	131.2	9.316	**
a	13	.9	1.4	61	136.8	144.8	11.131	**
i	12	36.7	50.7	61	55.0	108.4	.319	N. S.
Mama	13	247.7	82.0	59	139.0	105.1	11.924	**
Meme	13	287.3	89.4	60	189.3	127.5	6.757	*
Puppy	13	9.8	17.6	58	136.8	184.2	5.983	*
66	12	5.2	10.6	58	191.9	233.5	7.455	**
Kitty	12	6.7	13.1	58	166.0	174.5	9.705	**
Coca Cola	5	2.0	1.4	23	123.9	130.1	4.075	*

\* Significant at 5% level

\*\* Significant at 1% level

N. S. Not Significant

the airflow measured by the face mask equals that measured by the nasal and oral masks for these subjects.

#### *Airflow versus Judged Nasality*

Correlation coefficients between the airflow measurements for the cleft palate patients and the judged nasality were computed for the oral and nasal airflows. The results are shown in Tables 9 and 10. Only one sound, Coca Cola, showed significant correlation at the 5 percent level for the oral airflows. For the nasal airflows two sounds, (ū) and puppy, showed significant correlation with nasality at the 5 percent level and the two sounds, sixty-six and kitty, showed correlation at the 1 percent level.

#### *Simultaneous Oral Pressure and Nasal Airflow Measurements*

The readings obtained with the various pressure techniques and simultaneous measurements of the nasal airflow are shown in Table 11. During each test the patient was instructed to give maximum pressure. There is a definite

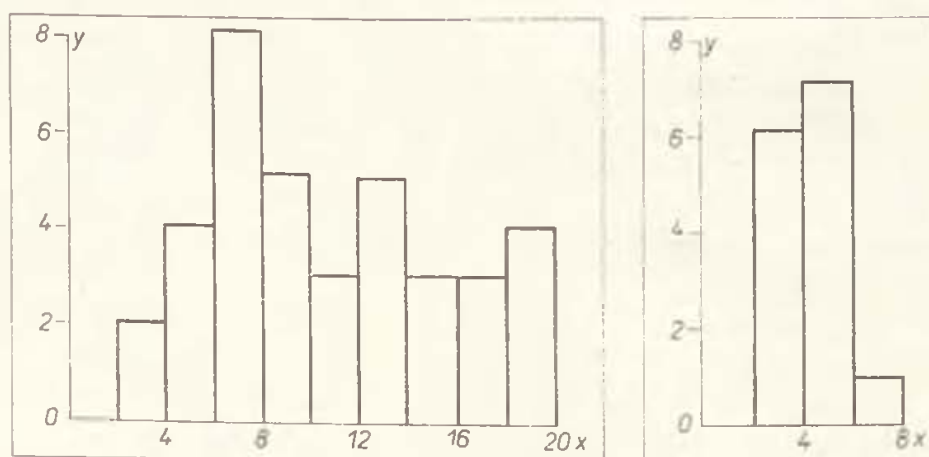


Fig. 5. Distribution of Judged Nasality. Cleft Palate Patients. — Fig. 6. Distribution of Judged Nasality. Normal Subjects. y — Number of Subjects

difference between the normal and cleft palate patients with respect to average pressure and flow. When the patient is using the Chase instrument and giving maximum expiration, there was no air leaking from the nasal cavity for the normal subject, and the average pressure was 53.2 oz. per inch squared. For the cleft palate patient, however, maximum expiration gave a pressure of 33.5 oz. per inch squared, and a nasal airflow of 626.5 ft. per minute. It will be observed that the same pattern found for the Chase Manometer was also observed to a lesser degree for the Hunter and Vital Capacity meters.

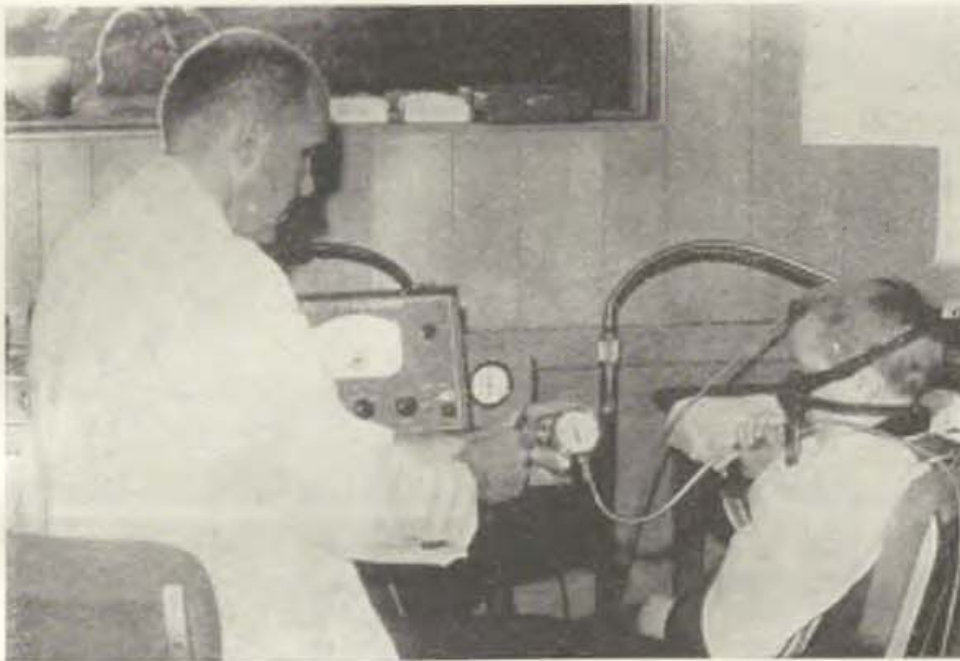


Fig. 7. Simultaneous Pressure and Airflow Measurements

The average pressures and average flows were compared separately for each group of subjects. No correlation between pressure and flow was found in any test for either group of subjects; the pressure developed during the test does not correlate with the nasal flow in any statistically significant way. In the case of the normal subjects tested with the Chase Manometer during expiration, the average flow was zero and a correlation coefficient could not be computed; this, however, was an exception.

*G. Correlation of Simultaneous Oral Pressure and Nasal Airflow with Judged Nasality*

The pressures and simultaneous nasal airflows that were obtained with the Chase, Hunter and Vital Capacity tests were correlated against the judged nasality for cleft palate subjects. The results are shown in Table 12. The data show that no significant correlations between pressure and judged nasality were obtained for any of the pressure techniques either on expiration or inspiration. All tests showed airflow on expiration had a significant correlation with judged nasality at the 5 percent level. The results from the Chase tests showed a correlation at the 5 percent level for the airflow during inspiration.

### *Flow Measurements*

The measurements of maximum airflow were undertaken to determine if there were a significant physiologic difference between the normal and cleft palate patients with respect to their ability to produce airflows. No significant differences were encountered between the two populations for this test. This is an indication that the ability to produce airflow was the same for both the normal and cleft palate patients studied.

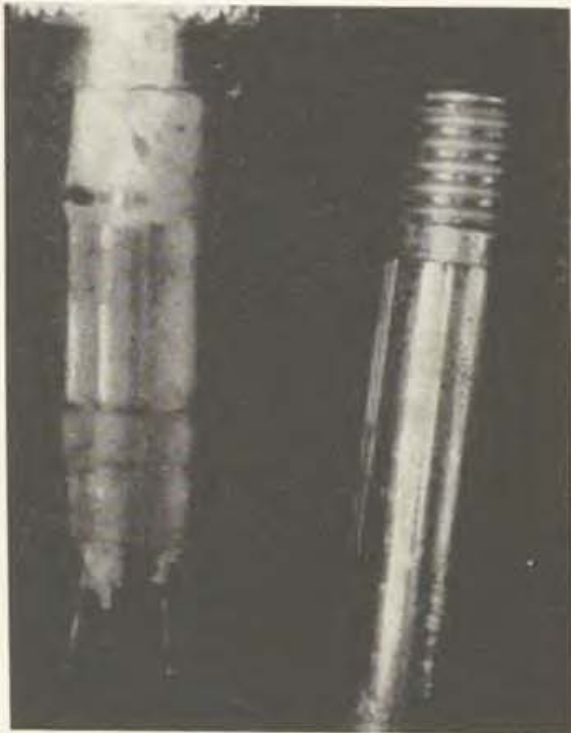


Fig. 8. Close up of „T“ Probe from Nasal Anemometer.

The foregoing conclusion is also substantiated by the data from measurement of the airflow during phrasing using the face mask. Here no significant difference was observed between the two groups.

With the oral mask significant differences in airflows were noted between the normal and cleft palate groups for the sound (ī), (ū) and (ā). These sounds can presumably be made with the velum raised or lowered although the normal position is with the velum lowered. The data show that oral airflow differs by almost a factor of two. Evidently the velum was raised for the cleft palate patients sufficiently to cause a substantial loss of air through the nasal cavity during the production of sustained vowels. Such changes were not seen in the same vowels when they were combined with consonants such as occur in mama, meme, puppy.

When the nasal mask was used, almost the reverse situation was observed. Significant differences in airflows were noted between the two groups. The most significant differences were found for the sustained vowels (ī) and (ū), as well as the phrases mama, sixty-six, and kitty. No significant difference existed for sustained (ā).



### Simultaneous Pressure and Flow Measurements

In both expiration and inspiration for normal and cleft palate patients, the same consistent pattern was observed for all instruments. In the normal subject when pressure was exerted upon the manometer little or no air escaped through the nasal port. For the cleft palate patient, however, less pressure was de-

Table 8. Correlation of the Facial Flow and the Sum of the Oral and Nasal Airflows  
Facial Airflow - (Oral Airflow + Nasal Airflow) =  $\Delta$  (Question is  $\Delta = 0$ )

Normal Subjects					
Sound	N.	$\Delta$	S. D.	T.	$\Delta = 0$
i	10	76.0	163.5	1.394	Yes
u	10	74.2	111.6	1.994	Yes
a	10	98.8	149.4	1.984	Yes
Mama	9	70.0	117.0	1.691	Yes
Meme	10	52.0	136.3	1.144	Yes
Puppy	10	37.8	72.6	1.562	Yes
66	9	38.1	108.2	.996	Yes
Kitty	9	35.3	109.8	.910	Yes
Coca Cola	4	2.8	48.8	.097	Yes

+ X = 0 at 1 percent level

Table 9. Correlation of Flow Measurements and  
Judged Nasality

#### Cleft Palate Patients

Oral Mask		
Sound	Number	Correlation Coefficient
i	18	.062
u	18	.150
a	17	.124
Mama	17	.339
Meme	18	.096
Puppy	18	.194
66	18	.333
Kitty	18	.054
Coca Cola	9	.468*

\* Significant at 5 percent level

veloped and substantial airflows were observed. An example is seen in Table 11 where for the normal patients a pressure of 53.2 oz. per in. squared was obtained by the Chase Manometer and no air escaped from the nasal port. In the cleft palate patients, however, the pressure was only 33.5 oz. per in. squared and air leakage was 626.5 ft. per minute. Similar results were obtained with other instruments.

It is often believed that increasing resistance to airflow tends to cause an increase in nasal air leakage. The resistances to airflow are ranked in terms of slight (Vital Capacity), moderate (Chase), and severe (Hunter). The average nasal airflows produced by the cleft palate patients during expiration and inspiration do not correlate in this order, but in the order of slight (Vital Capacity), severe (Hunter), and moderate (Chase). The spreads in the average airflows of the cleft palate group, which are indicated by their standard deviations, are so large that no statistical conclusion can be attributed to this reversal in their ranking.

The most significant result from this part of the study is the discovery that there is no correlation between the pressures developed by the cleft palate patients and the concurrent nasal air leakage. (Table 11.)

Table 10. Correlation of Flow Measurements  
and Judged Nasality  
Cleft Palate Patients

Nasal Mask		
Sound	Number	Correlation Coefficient
i	20	.348
u	20	.504*
a	20	.127
Mama	19	.115
Meme	20	.196
Puppy	19	.420*
66	20	.560**
Kitty	20	.556**
Coca Cola	7	.008

\* Significant at 5 percent level

\*\* Significant at 1 percent level

#### *Correlation of the Various Measurements with Judged Nasality*

The correlation of the measurement techniques with judged nasality may be summarized as follows: no significant correlations between judged nasality and readings from the various pressure measuring techniques, using the Hunter, Chase, or Vital Capacity instruments, was found when simultaneous oral pressure and nasal airflow were measured. However, the nasal airflow produced when exerting pressure into the instruments did correlate at the 5 percent level with judged nasality. This correlation between flow and nasality occurred for all pressure methods during expiration and with the Chase data during inspiration. Airflow measurements during phrasing of speech sounds also showed correlation with judged nasality. This correlation was most evident with the nasal airflow measurements. The oral airflow measurements showed correlation for one phrase "Coca Cola" at the 5 percent level. The nasal airflow measurements showed correlations with judged nasality at the 5 percent level for the

sustained vowel [u] and the word puppy, and at the 1 percent level for the sounds "sixty-six" and "kitty".

The lack of correlation between most of the various measurements and the judged nasality is a direct indication of the complexity of the nasality concept. Only the sounds "mama" and "meme" of the nine test phrases involve a deliberate lowering of the velum and consequent opening of the nasal cavity. Airflow measurements for these phrases showed no correlation with judged nasality. According to Heffner (8) nasality is produced only by a vigorous lowering of the velum and a deliberate construction of the posterior pillars of the fauces. The position of the velum is not critical for the vowel sounds, sustained [i], [u], [a], which can be produced adequately through compensatory muscular movements with the velum open or closed [8, 12]. Presumably cleft palate patients have learned to make such compensatory movements and

Table 11. Correlation of Simultaneous Oral Pressures and Nasal Airflow for Normal and Cleft Palate Patients

	N	M	S. D.	Corr. Coeff.	N	M	S. D.	Corr. Coeff.
Expiration								
Hunter Flow	10	15.8 0.2	2.6 0.6	.1819	36	10.6 575.5	5.2 795.0	.2305
Inspiration								
Hunter Flow	10	14.9 0.9	3.8 2.1	.4939	27	13.1 487.3	24.4 608.4	.0820
Expiration								
Chase Flow	5	53.2 0.0	7.9 0.0		28	33.5 626.5	16.8 736.2	.0446
Inspiration								
Chase Flow	5	57.6 1.6	7.2 2.1		28	25.3 638.3	17.5 699.0	.1662
Expiration								
Vital Capacity Flow	8	29.0 1.8	5.4 3.6	.1820	27	22.9 427.0	11.9 454.7	.1890

Table 12. Correlation of Simultaneous Pressure and Flow Measurements to Judged Nasality

		N	Coefficient
Hunter			
Expiration	Pressure	16	0.2197
	Flow	16	0.5235*
Inspiration	Pressure	16	0.1928
	Flow	16	0.3814
Chase			
Expiration	Pressure	10	0.1027
	Flow	10	0.6939*
Inspiration	Pressure	10	0.2307
	Flow	10	0.6892*
Vital Capacity			
Expiration	Pressure	13	0.2689
	Flow	13	0.5576*

\* Significant at 5 percent level

in fact can eliminate nasality by deliberate counter movements of the muscles of the oral cavity for many similar sounds. Their failure to prevent the unwanted appearance of nasal sounds seems to occur under the following conditions: (1) muscle fatigue, such as caused by sustained discourse, (2) the production of unfamiliar sounds, (3) when action are required which make compensation difficult, such as the control of airflow during the pressure measurements.

A great amount of work needs to be done in the field of objective testing. The present data show heartening trends indicating that objective clinical tools for the assessment of velar-pharyngeal competence can be found. The present information shows that the nasal airflow meter may be a useful clinical tool although much more needs to be learned about its proper application.

#### SUMMARY

This report summarizes results from a larger study directed toward the development of practical clinical instrumentation to measure palatopharyngeal competence. The relationship between airflow during speech and judged nasality has been examined.

Several important results were derived from direct airflow measurements. (1) No significant difference occurred between the total air expelled through a face mask for either the normal or cleft palate subjects speaking the nine test phrases or for maximum airflow. (2) Differences between the airflow characteristics of the two groups first appeared for the oral airflows associated



with the emission of the sustained vowels [ī], [ū], [ā]. Significant differences were observed between normal and cleft palate groups for the nasal airflows associated with eight of the nine test phrases. (3) The only test phrase not showing a significant difference between the nasal airflow of the normal and cleft palate patients was the sustained vowel [ā].

In simultaneous air pressure and airflow measurements, it was determined that for the normal subject there is little or no nasal air leakage in the presence of high oral pressures. In the cleft palate patient, however, on the average lower oral pressures were accompanied by dramatically high nasal airflows. It is even more important to point out that there was no correlation between the oral pressures and nasal leakage for individual cases. Should this result be substantiated by more extensive studies with a larger cleft palate population, the usefulness of the pressure testing techniques would seem open to serious doubt.

The results from the measurements of direct airflow and the measurement of simultaneous oral and nasal airflow have been correlated with judged nasality. Judged nasality correlated significantly with nasal airflows during the test phrases sustained [ū], puppy, sixty-six and kitty.

Judged nasality also had a statistically significant correlation with the nasal airflows which occurred during pressure testing.

No statistically significant correlation was found at any time between judged nasality and the results of any pressure tests made during simultaneous measurements of nasal airflow and oral pressure.

## R É S U M É

### Une évaluation clinique et subjective de la parole

L. F. Quigley Jr., C. M. Cobb

Ce travail résume les résultats d'une expertise visant le développement d'une instrumentation clinique servant au mesurement de la compétence palatopharyngienne. La relation entre le courant d'air au cours de la parole et la nasalité jugée a été soumise à l'examen.

A l'aide d'un mesurement direct du courant d'air respectif on a gagné quelques-uns des résultats très importants.

1. Il n'y avait aucune différence significative entre le total au maximum de l'air expulsé à travers le masque facial chez les individus au palais normal et ceux à la fente palatine prononçant neuf des mots de teste.

2. Les différences dans la caractéristique du courant d'air chez les deux groupes se montrèrent d'abord chez le courant d'air oral en relation avec la prononciation des voyelles prolongées [ī], [ū], [ā]. Les différences significatives ont été trouvées chez les individus normaux au cours de la prononciation des huit des neuf mots de teste.

3. L'unique mot ne prouvant aucune différence significative dans le courant d'air nasal entre les personnes normales et ceux à la fente du palais était la voyelle prolongée [ā].

Au cours du mesurement simultané de la pression de l'air et du courant d'air on a trouvé le fait que chez les personnes normales la fuite d'air à travers le nez vis-à-vis d'une pression augmentée de l'air dans la bouche est minimale ou bien encore nulle.

Chez les personnes souffrant de la fente du palais la pression de l'air réduite dans la bouche se trouve, au contraire, accompagnée du grand courant d'air dramatique dans le nez.

Si nous arrivions à confirmer ce résultat par des épreuves sur un assemblément significatif des personnes à la fente du palais, on pourrait très sérieusement se douter du prix des méthodes de teste de la pression. Les résultats obtenus à l'aide du mésurement direct du courant d'air sous mésurement simultané du courant d'air dans le nez et dans la bouche ont été mis en relation avec la nasalité jugée. Cette nasalité jugée était en corrélation significative avec le courant d'air des mots de teste à la voyelle prolongée [ū] — puppy, sixty-six and kitty. La nasalité jugée montrait de même une relation significative quand à la statistique envers le courant d'air nasal formé au cours des testes de la pression. Entre la nasalité jugée et les résultats des testes de la pression mis en pratique au cours des mésurements du courant d'air nasal et la pression de l'air on n'a jamais trouvé une corrélation significative quand à la statistique.

## ZUSAMMENFASSUNG

### Klinische und subjektive Auswertung der Sprache

L. F. Quigley, Jr., C. M. Cobb

In der vorliegenden Arbeit werden die Ergebnisse einer umfangreicheren, auf die Entwicklung des praktischen klinischen Instrumentariums zur Messung der palatopharyngealen Zugehörigkeit eingestellten Studie zusammengefasst. Es wurden Untersuchungen über die Beziehung zwischen der Luftströmung beim Sprechen und der beurteilten Nasalität durchgeführt.

Durch direkte Luftströmungsmessungen wurden einige bedeutende Ergebnisse gewonnen. 1. Zwischen der gesamten, durch die Gesichtsmaske ausgetriebenen Luftmenge, die bei Personen mit normalen Gaumen und Individuen mit Gaumenspalte bei der Aussprache von neun Testwörtern ermittelt wurde, und der maximalen Luftströmung sind keine signifikanten Unterschiede festgestellt worden. 2. Unterschiede im Charakter der Luftströmung für diese zwei Gruppen erschienen in der oralen Luftströmung bei der Aussprache von prolongierten Selbstlauten [ī], [ū], [ā]. Signifikante Unterschiede erschienen zwischen normalen Individuen und Personen mit Gaumenspalte bei normaler Luftströmung bei der Aussprache von 8 aus 9 Testwörtern. 3. Das einzige Testwort, bei dem kleine signifikanten Unterschiede zwischen der nasalen Luftströmung bei normalen Individuen und Personen mit Gaumenspalte ermittelt worden sind, war der prolongierte Selbstlaut [ā].

Bei gleichzeitiger Messung des Luftdrucks und der Luftströmung wurde ermittelt, dass bei normalen Personen der Luftverlust durch die Nase in der Anwesenheit von hohem oralen Luftdruck gering ist oder Null gleicht. Bei Personen mit Gaumenspalte wird dagegen im Durchschnitt der niedrigere orale Luftdruck von einem dramatisch hohen Anstieg der nasalen Luftströmung begleitet. Es ist sogar noch wichtiger, wenn betont wird, dass zwischen dem oralen Luftdruck und dem nasalen Luftverlust in einzelnen Fällen keine Korrelation vorzufinden war. Sollte dieses Ergebnis durch weitere umfangreichere Studien an einer grösseren Anzahl von Personen mit Gaumenspalte bestätigt werden, dann würde der Wert der Luftdrucktestungstechnik ernststen Zweifeln ausgesetzt sein.

Die Ergebnisse der Messung der direkten Luftströmung und der Messung der simultanen oralen und nasalen Luftströmung wurden mit der beurteilten Nasalität in Korre-

lation gestellt. Die beurteilte Nasalität wies mit der nasalen Luftströmung bei der Aussprache von Testwörtern mit prolongiertem [ū], und von puppy, sixty-six, und kitty eine signifikante Korrelation auf.

Die beurteilte Nasalität war auch in statistisch signifikanten Verhältnis zu den nasalen Luftströmungen, die bei der Drucktestung erschienen sind.

Zwischen der beurteilten Nasalität und den Ergebnissen der Drucktestung bei gleichzeitiger Messung der nasalen Luftströmung und des oralen Druckes ist keine statistisch signifikante Korrelation ermittelt worden.

## RESUMEN

### Evaluaciones subjetivas y clínicas del lenguaje

L. F. Quigley, hijo., C. M. Cobb

Este artículo hace un sumario hecho a base de un estudio más extenso referente al desarrollo de los instrumentos clínicos para medir la capacidad palatofaríngea. La relación entre el flujo del aire durante el lenguaje y el sonido nasal ha sido examinada.

Diversos resultados importantes fueron obtenidos a base de la medición directa del flujo del aire. 1. No ocurrió una diferencia grande entre el volumen total del aire expulsado por la careta del rostro ni en las personas con el paladar normal o paladar fisurado diciendo las nueve frases de pruebas ni en el caso de un flujo del aire máximo. 2. Diferencias entre las características del flujo del aire en los dos grupos aparecieron por la primera vez en los flujos del aire orales acompañados de la emisión de las vocales sostenidos, es decir en [ī], [ū] y en [ā]. Diferencias significantes se observaron entre los grupos normales y los con el paladar fisurado como el flujo del aire nasal fue presente en ocho de las nueve frases de pruebas. 3. La sola frase de pruebas que no demostró una diferencia significativa del flujo del aire entre los pacientes normales y los con el paladar fisurado contuvo la vocal sostenido [ā].

En las presiones del aire simultáneas y en la medición del flujo del aire se fijó que en los pacientes normales ocurrió un poco o ningún escape del aire nasal en el caso de grandes presiones orales. Pero en los pacientes con el paladar fisurado por término medio las presiones orales poco elevadas fueron acompañadas de los flujos del aire considerablement elevados. Es aún más importante acentuar que no haya correlación alguna entre las presiones orales y el escape nasal en casos individuales. Si este resultado sea substanciado por los estudios más extensos referentes a los casos con un paladar fisurado más grande, la utilidad de las técnicas de presión experimentales pueda hacerse dudable.

Los resultados obtenidos a base de la medición del flujo del aire directo y la medición del flujo del aire simultáneo, desde el punto de vista oral y nasal, fueron puestos en correlación con la calidad nasal. Esta tuvo una correlación significativa con el flujo del aire nasal durante las frases de pruebas sostenidas [ū], es decir „puppy“, „sixty-six“ y „kitty“.

La calidad nasal tuvo también una correlación muy significativa desde el punto de vista estadístico con los flujos del aire nasales que tuvieron lugar durante el test de presión.

Ninguna correlación que sea significativa desde el punto de vista estadístico se halló en modo alguno entre la calidad nasal y los resultados de todos los experimentos de presión hechas durante la medición simultánea del flujo del aire y de la presión oral.



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## ACTION POTENTIALS OF LEVATOR AND TENSOR MUSCLES IN PATIENTS WITH CLEFT PALATE

I. ŠURINA, J. JÁGR

The original role of the palate was to separate the oral digestive tract and the pharynx from the nasal passages. The palato-pharyngeal closure occurs as early as during the first cry of the new-born infant, as well during the suction of maternal milk.

The speech represents phylogenetically the youngest function and the activity of the palato-pharyngeal sphincter during articulation is only secondary. In the intercommunication between humans the speech fulfils a mission which is specific for human beings [7]. A normal individual, by means of the control exerted by the central nervous system and the senses, particularly hearing, regulates the amount of sound entering into his nasal resonant cavities and this provides the speech with an appropriate aesthetic colouring. The formation of mutual relations of the resonant cavities and their automatic



Fig. 1a. The introduced electrodes A: into the m. levator v. p., B: into the m. tensor v. p. A normally developed soft palate from the group of individuals without speech disorder.

modulation and configuration begins as early as in infancy. Thus develops the stereotype of speech. The jaw-angle, the modulation of the shape of the cavities above the vocal cords by the muscles of the tongue, the lips, soft palate, pharyngeal and laryngeal muscles, all of them participate in the formation of sounds during articulation.



Fig. 1b. The introduced electrodes A: into m. levator v. p., B: into m. tensor v. p. Palate from patient with a cleft after surgical repair (short hard and soft palate, atrophic praemaxilla).

The maximum relaxation of the velopharyngeal sphincter occurs during the pronunciation of the so called nasals, e.g. m, n, ng, while the pronunciation of other letters is associated with varying degrees of contraction resulting in a more or less marked palato-pharyngeal closure (7,8).

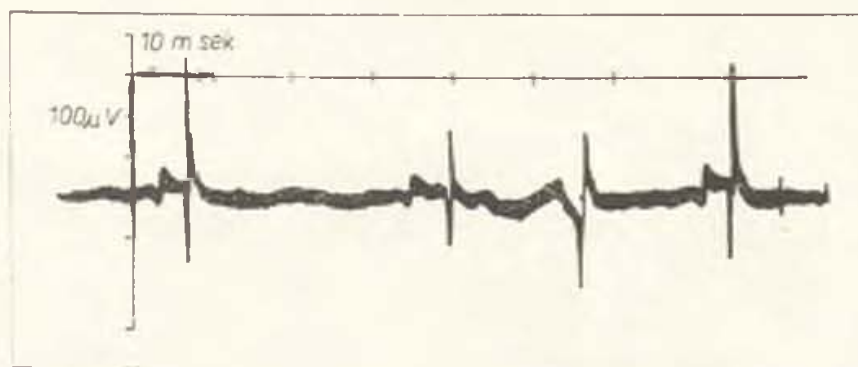


Fig. 2a. EMG pattern of the m. tensor v. p. in a group with a normal palate.

The physiologic autoregulation of a correct articulation is markedly disturbed in patients with cleft palate. Individuals with this defect compensate and obturate the objective deficiency of the peripheral speech organ with the help of groups of muscles with a higher motoric efficiency, in the aim to obtain either a normalization or at least an improvement of function.

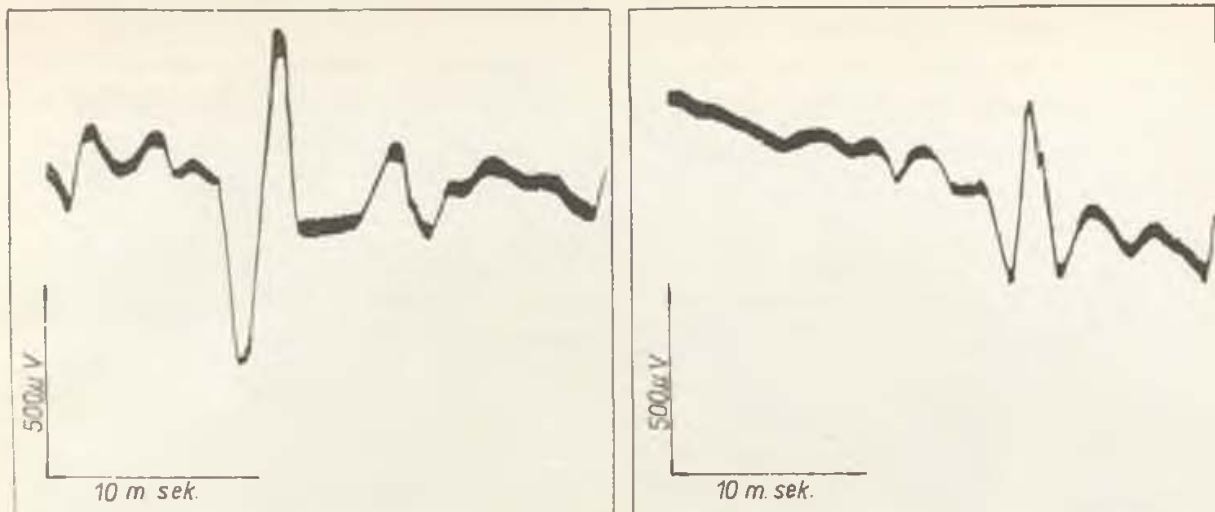


Fig. 2b. EMG pattern of the m. levator v. p. from the group with a normal palate. —  
Fig. 2c. EMG pattern of the m. lev. v. p. from the group with a normal palate.

In a patient with cleft palate there is a disorder of the speech, involving the resonant, acoustic and articulatory components. In spite of the typical connection between this speech disorder and the insufficiency of the velopharyngeal sphincter, it is designated as rhinolalia. An excessive amount of sound energy enters into the nasal passages resulting in a unaesthetic type of rhinophonia due to the aperture. The cleft palate causes the fusion of the resonant oral and nasal cavities (2, 5, 6, 9). Conceivably the type of the cleft characterizes and influences the quality of speech disorders.

Various techniques of surgical repair are used in the aim to create the prerequisites of a correct articulation, as well as an appropriate colouring of the speech.

In practice we encounter cases, however, in whom similar inborn defects of the peripheric speech organ treated with an identical surgical method fail

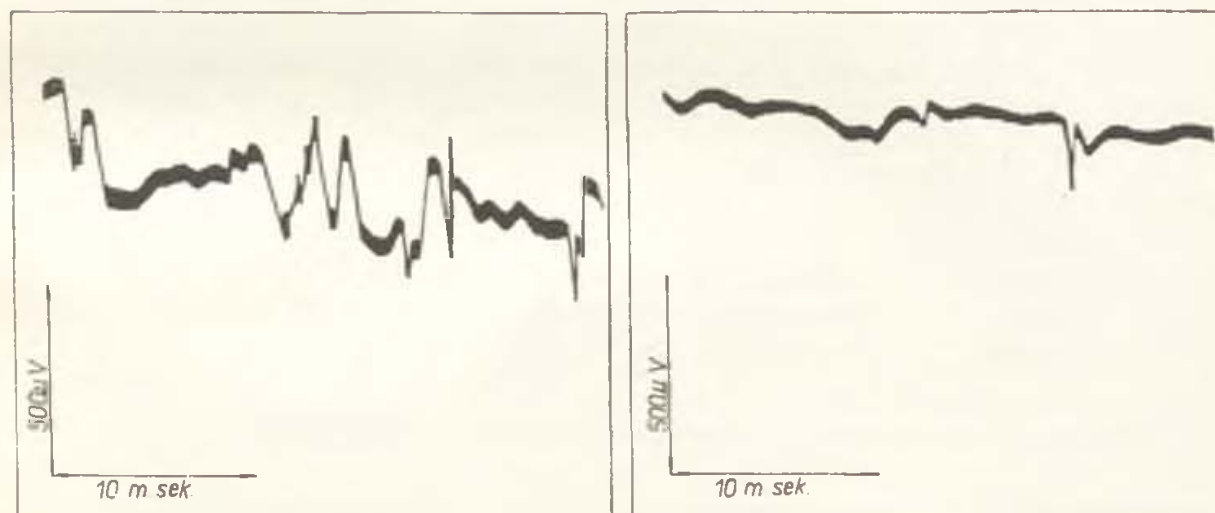


Fig. 2d. EMG pattern of the m. lev. v. p. from the group of patients with clefts. —  
Fig. 2e. EMG pattern of the m. lev. v. p. from the group of patients with clefts.



to obtain identical functional results with regard to the speech. Essential factors influencing the character of a typically impaired speech in patients with clefts include the motoric quality of the muscles of the velo-pharyngeal sphincter, since its function represents a component of the coupled system of voice and speech formation. The velo-pharyngeal sphincter plays an important part in the quality of the resonance of formed vocals and articulated sounds. The

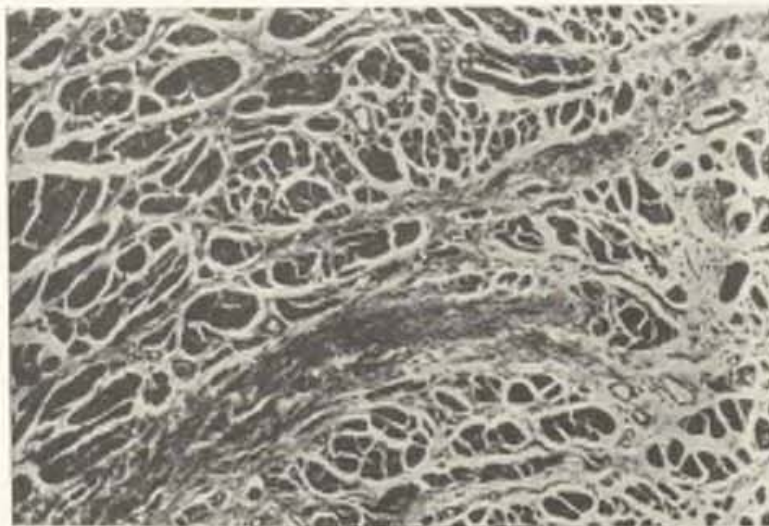


Fig. 3a. Muscles of a normal soft palate (sections obtained from a fresh corpse) (sectioned across the muscle bundles).

refined co-ordination of the motoric function of the soft palate during both swallowing and speech encloses all muscles into one functional unity.

Electromyography of the soft palate was used for the study of the morphology of action potentials of the two most characteristic levator and tensor muscles in normal individuals and in patients with cleft palate. The results of the electromyographic study were compared with the histologic pattern of small specimens excized during the surgical repair of cleft palate, as well as with specimens of muscles from normal and cleft palate obtained from fresh corpses autopsied at the Institute of Pathologic Anatomy at Bratislava.

#### MATERIAL AND METHODS

Our patients were subdivided into two groups. The first group included 7 patients with normal palate development. Their age ranged from 6 to 20 years. In the second group were 11 patients with cleft palates both prior to and after surgery, and ranging in age from 6 to 55 years. Their soft palate showed a reduced mobility and a more or less marked rhinolalia was present. Prior to the introduction of the needle electrodes the mucous membrane of the palate was anaesthetized with a percent mesocain spray. The musculus levator veli palatini was inserted with electrodes about 3—4 mm medially from the hamulus pterygoideus in dorso-cranial direction with medial deviation about  $15^{\circ}$ , and into the depth of 1.5 cm (according to the character of the

palate). M. tensor veli palatini was inserted about 3—4 mm laterally from the hamulus pterygoideus in cranial direction with a slight dorsal deviation into the depth of about 1.5 cm. In patients with cleft palates the introduction was adjusted according to the situation of the soft palate which is mostly shorter and has less muscle tissue. As the orientation point served a small excavation observed during the contraction of the soft palate at the time of the insertion



Fig. 3b. Muscles of a normal soft palate [sections obtained from a fresh corpse] [sectioned both across and alongside the muscle bundles].

into the m. levatoris veli pal. [see Fig. 1a, 1b: introduction of the electrodes in to the levator (A) and tensor (B) in a normal palate and in a patient with cleft palate]. The examination was carried out with a 3 channel electromyographic apparatus "Disa" with the used of the above mentioned unifilar needle electrodes. Action potentials of the muscles were registered on photographic paper by means of a transversal recording, with the speed of light of 1 mm/msec. (1 m/sec). The amplification resulted in a deviation corresponding to 50 microvolts. The duration and amplitude of the potentials in the two groups were statistically evaluated with the t test. Both the amplitude and the duration of 50 potentials were assessed in each individual examined. In some cases the electromyographic curve was constructed as well, by continuous recording at a speed of the paper of 20 cm/sec. In occasional cases a 2 channel apparatus "Disa" was used for the recording. See Fig. 2a: a tensor muscle (in a normal individual), 2b, 2c: m. levator (in a normal individual) Fig. 2d, 2e: m. levator (in a patient with cleft palate). The histologic study of the specimens which was carried out only with regard to the basic problem included 5 specimens excised peroperatively from patients with cleft palate approximately from the same site. The specimens were immediately after excision placed into a solution of alcohol and formol. Specimens of the soft palate muscles obtained from fresh corpses (2 excisions from clefts and 3 excisions from normal palates). The specimens were placed in formol (1:4) and examined at the Institute of

Histology and Embryology in Bratislava. After fixation, embedding and sectioning the specimens were stained with hematoxylin-eosin, orceine, with trichrome according to Masson, impregnated for neurofibrils according to Holmes and with the use of PAS. (Technical co-operation: M. Lednářová, laboratory worker).

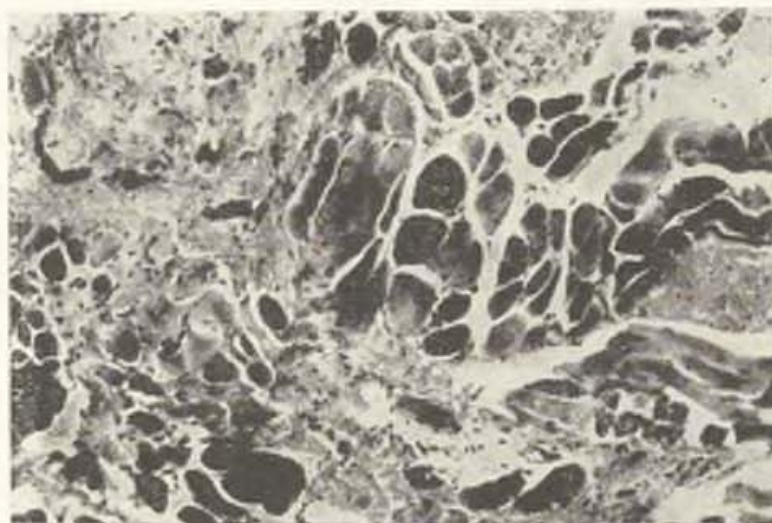


Fig. 3c. Muscles from a patient with cleft palate. (Rarefaction of the muscles containing numerous large bands of connective tissue.)

#### RESULTS

According to our results in the group without cleft and without speech disorders the mean duration of potentials was 3.6 msec. The mean duration of potentials in patients with cleft palate and with speech disorders was 2.4 msec. This difference is statistically significant ( $P < 0.01$ ). The median amplitude of potentials in the groups of normal peoples was 320 microvolt, while in the group with cleft palate it was 235 microvolts. The difference of the median amplitude in both groups is statistically significant ( $P < 0.01$ ). This fact is further substantiated by the histologic pattern of the specimens. The characteristics of muscles from patients with cleft palate differ from that of the normal muscles from the same site; individual muscle layers are less closely packed together, include a relatively scarce number of muscle fibers separated by large bands of connective tissue. The contractile substance is reduced and replaced by connective tissue. See Fig. 3a, 3b: muscles of a normal soft palate. Fig. 3c, 3d: muscles of the soft palate from a patient with cleft palate. (Photo: Ultraphot Zeiss, ocular 10, object-glass 10, J. Uher.)

#### DISCUSSION

The electromyographic values obtained in normal individuals are in agreement with the data reported by G. Böhme, F. Šram and E. Kalivodová who studied the levator and tensor muscles during respiration and phonation (1). The action potentials determined in our series and those observed by the above



mentioned authors, however, were of longer duration compared to the data ascertained by Cho-Lu-Li and Lundervold [4].

A significant shortening of action potentials duration, as well as a reduction of their amplitude resembled to the electromyographic pattern observed in primary muscular disorders [10].

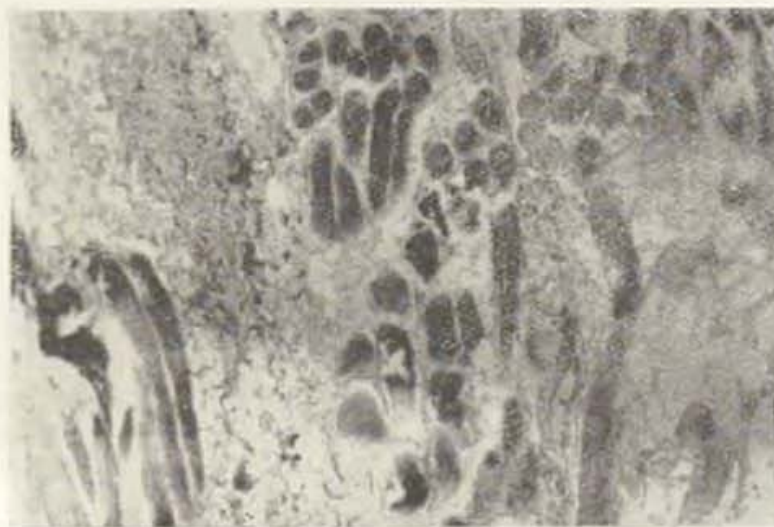


Fig. 3d. Muscles from a patient with cleft palate. A reduced amount of muscle tissue separated by numerous bands of connective tissue.

On the base of the electromyographic pattern it is therefore possible to assume that, at least some of the patients with cleft palate besides other muscle changes have a primary lesion of muscle fibers as well. This assumption is formulated with caution because of the fact that in the group of patients with cleft palate we failed to demonstrate in some cases a shortening of the action potentials duration.

#### SUMMARY

The authors used electromyography in 18 individuals for the study of the mm. levator et tensor veli palatini (7 normal individuals and 11 patients with cleft palate). The patients examined had a typical speech disorder of varying degree. In clinically similar cases of insufficiency of the velopharyngeal sphincter fail to show a direct relation to the characteristic of the functional speech disorder. It is conceivable that, besides the type and form of the developmental disorder, many factors influence the character of the speech disorder. Patients with cleft palate showed before and after the operation a significantly lower median amplitude of action potentials, i. e. 235 microvolts, compared to 320 microvolts in normal individuals. The mean duration of action potentials in patients with cleft palate was statistically highly significant (2.4 msec) compared to the values observed in a group of normal individuals. These results are in agreement with the histologic pattern of a normal and of a cleft palate. The rarefication of the muscles in patients with clefts is due to the replacement of the contractile muscle substance by connective tissue.

The characteristic of the electromyographic pattern shows a good agreement with the character of the functional speech disorder.

The present paper is aimed at a stimulation of further detailed study of the qualitative and quantitative character of the muscles of the soft palate in patients with clefts, according to the type of cleft and to the influence of the surgical technique on the muscles of the soft palate.

## R É S U M É

### **Les potentiels de l'action musculaire des releveurs et du muscle péristaphylin externe chez les malades souffrant de la fente du palais**

I. Šurina, J. Jágr

Les auteurs ont examiné à l'aide de l'électromyographie chez 18 des personnes le releveur du voile du palais de même que le muscle péristaphylin externe. Sept de ces personnes étaient de contrôle, onze souffraient de la fente du palais. Ceux-ci présentaient un certain défaut de la prononciation plus ou moins exprimé. Dans la clinique les cas pareils de l'insuffisance du muscle péristaphylin externe ne présentent aucune dépendance directe avec le caractère de l'endommagement de la fonction de la parole.

Donc, le caractère de l'endommagement de la prononciation dépend de plusieurs facteurs à part le type et l'aspect du défaut de l'organe de la parole lui-même. Chez les malades à la fente du palais parfois avant parfois même en suite de l'intervention chirurgicale l'amplitude médiale des potentiels se tenait aux 235 mV — ce que représente un abaissement significatif en relation avec 320 mV de la groupe de contrôle. Le temps médial de la durée des potentiels des malades à la fente du palais était d'une grande signification à l'égard de la statistique (2,4 msec) en relation avec la groupe de contrôle (3,6 msec).

Ces résultats sont d'accord en comparaison avec les données histologiques du palais parfait et celui à la fente. La raréfaction de la musculature chez les malades à la fente du palais était remplacée par le tissu ligamentaux faute du matériel contractif musculaire.

Le caractère des données EMG était en relation avec le caractère du défaut de la fonction de la parole.

Le travail a mis en réalisation une étude très détaillée du caractère en tant que qualitatif de même que quantitatif de la musculature de la voile du palais chez les malades à la fente du palais. D'après le type de la fente et l'influence de l'intervention en question touchant la musculature de la voile du palais.

## ZUSAMMENFASSUNG

### **Muskelaktionspotentiale des Levators und Tensors bei Kranken mit Gaumenspalte**

I. Šurina, J. Jágr

Die Autoren untersuchten elektromyographisch die Mm. levator et tensor veli palatini bei 18 Individuen (7 gesunde Individuen und 11 Kranke mit Gaumenspalte). Bei den untersuchten Kranken war eine typische Sprachstörung eines grösseren oder geringeren Umfanges festzustellen. Klinisch ähnliche Fälle der Insuffizienz des velopharyngealen

Sphinkters stehen nicht in direkten Zusammenhang mit dem Charakter der Schädigung der Sprachfunktion. Es ist klar, dass der Charakter der Sprachfunktion — ausser des Typs und der Form der Entwicklungsstörung des peripheren Sprachorgans — von mehreren Faktoren beeinflusst wird. Bei Patienten mit Gaumenspalte war die mittlere Amplitude der Potentiale vor sowie auch nach der Operation signifikant niedriger (235 Mikrovolt) im Vergleich mit der Gruppe der gesunden Individuen (320 Mikrovolt). Die durchschnittliche Dauer der Potentiale war bei den Kranken mit Gaumenspalte statistisch hoch signifikant (2,4 msec) im Vergleich mit der Gruppe der gesunden Individuen (3,6 msec). Die Ergebnisse stehen in Übereinstimmung mit dem Vergleich zu der Histologie des gesunden und gespalteten Gaumens, die Verdünnung des Muskelgewebes bei Kranken mit Gaumenspalte wurde ersetzt durch Bindegewebe zum Nachteil der kontraktilen Muskelmasse.

Der Charakter des elektromyographischen Bildes war in Übereinstimmung mit dem Charakter der funktionellen Sprachstörung.

Diese Arbeit ist Anregung zum begonnenen eingehenderen Studium des qualitativen und quantitativen Charakters des Muskelgewebes des weichen Gaumens bei Patienten mit Gaumenspalte je nach dem Typ der Spaltung und des Einflusses der Operationsmethoden auf das Muskelgewebe des weichen Gaumens.

## RESUMEN

### **Los potenciales musculares de acción del levador y del tensor en los pacientes con la grieta del paladar**

I. Šurina, J. Jág r

Los autores examinaron electromiográficamente en 18 individuos mm. levator et tensor veli palatini (7 individuos sanos y 11 pacientes con la grieta del paladar). En los pacientes examinados se hallaba un defecto típico de lenguaje del grado mayor o menor. Clínicamente los casos semejantes de la insuficiencia del sphincter velopharyngealis no son en la relación directa con el carácter del daño de la función de lenguaje. Es claro, que el carácter del defecto de lenguaje tiene la insuficiencia sobre más factores con excepción del tipo y de la analogía del defecto de desarrollo del órgano periférico de lenguaje. En los pacientes con la grieta del paladar sea antes de la operación, sea después de ella, la amplitud central de los potenciales fue significativamente más baja, 235 microvoltios en comparación de un grupo de sanos 320 microvoltios. El tiempo medio de la duración de los potenciales en los pacientes de grieta fue estadísticamente muy significativo (2,4 msec) en comparación de un grupo de sanos (3,6 msec). Los resultados se dan por entendido con la comparación con la histología del paladar sano y el de grieta. La aclaración de la musculatura en los pacientes de grieta fue reemplazada con el ligamento en perjuicio de la substancia muscular contráctil.

El carácter del cuadro EMG se dió por entendido con el carácter del defecto funcional de lenguaje.

Este trabajo es un impulso al estudio empezado más detallado sobre el carácter cualitativo y cuantitativo de la musculatura del paladar blando en los pacientes de grieta según el tipo de la grieta y según la influencia de los métodos operatorios a la musculatura del paladar blando.

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## RECONSTRUCTIVE PLASTIC SURGERY IN EXTENSIVE PERFORATIONS OF THE NASAL SEPTUM

### Personal Technique\*)

A. MARINO

It is well known that extensive perforations of the nasal septum are always severely disturbing the patients. The patients' complaints vary and depend partly on the extent of the defect, partly on the anatomical and functional situation of the turbinates the volume changes of which are modified by specific physiological and environmental influences which interfere with the nasal respiratory quotient.

If therefore a change in the patency of nasal passages occurs in subjects suffering from chronic hypertrophic rhinitis a very disagreeable "cross ventilation" is caused. This will happen still more frequently in persons suffering from a severe progressive atrophic crustous rhinitis which is usually complicated by cacosmia, rhinopharyngitis and a catarrhal tubotympanic inflammation, particularly when under serious endo- or endogenous influences. The crusts situated often marginally along the edges of the defects cause, when getting loose, frequent and repeated epistaxis.

If there are large perforations situated at the level of the anterior cartilaginous arc in the direction of the nose ridge, functional changes are accompanied by a serious cosmetic defect, either a deflected nose tip or a secondary saddle deformation of the superior cartilaginous arc.

Besides various pathological causes of such septum perforations, the main agent in the origin of perforations are surgical endonasal interventions, either of a therapeutic character — cautery of "locus Valsalvae" in recurrent epistaxis — or of a corrective character aiming at the remedy of morphological anomalies of the septum.

Reconstruction of the septum, in cases of small perforations, is usually successful if the so called "French" or "V—Y" technique is used.

On the other hand, medium-sized perforations, as pointed out by Scott-Brown, are not always manageable by reparatory plasty. Some of these re-

\*) Presented at the IVth Internat. Congress on Plastic Surgery, Rome 8—13th October 1967.



constructive techniques may be used only under particular anatomical situations which allow their performance, as is the case with Seiffert's Aubry-Garez's technique etc. There are other techniques (for instance Climo's the Indian method of septoplasty) which are rather exacting, and often do not guarantee a safe result.

In cases of large perforations of the septum it is necessary to recall Ballengier's authoritative statement, made already in 1943, that surgically they are irreparable. According to Aubry and Garez tubed-flap grafts from more remote

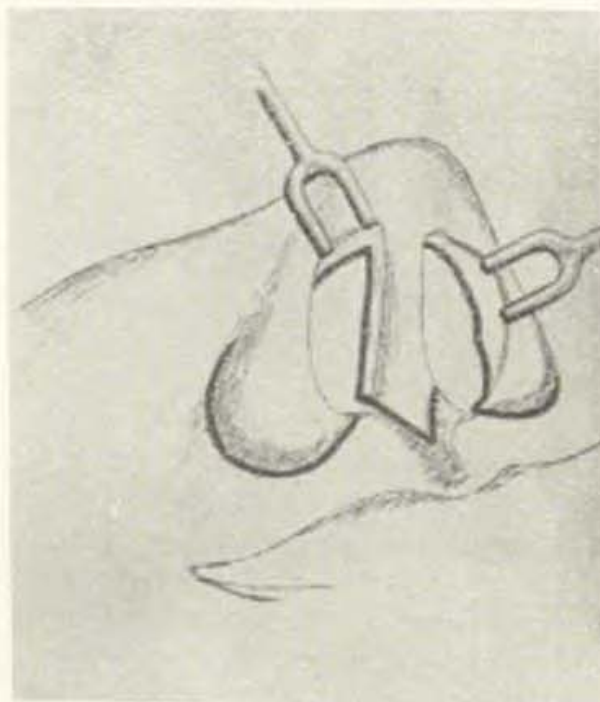


Fig. 1.



Fig. 2.

sites would be indispensable for their reconstruction. It is superfluous to emphasize that, considering the long series of surgical stages and the relative damage to the patient, the common transvestibular approach is a very difficult procedure even with the use of a sparing technique.

In 1966 already, we proposed a reconstructive technique aiming at an easier repair of medium and extensive perforations of the nasal septum.

This method was improved later on and it allows, without any risky interventions, a reconstruction of extensive defects which could not have been corrected by the methods proposed until now.

#### TECHNIQUE

The intervention consists of seven surgical stages which are performed in local infiltration anaesthesia after appropriate premedication; it is better still to use "limited anaesthesia" or "dip-dol" anaesthesia.

It is very important to be sure that the anaesthetic solution is injected under the mucous membrane of the nasal cavity so as to attain an easier

gradual preparation of this mucosa, a correct performance of this manoeuvre is the key to the successful performance of the whole operation.

*Stage 1: Section of the base of the pyramid by external dissimulated incisions. [Fig. 1.]*

The nostrils are separated by transfixion along the sulcus alaris. These incisions are further elongated into the nasal meatus between the anterior margin of the triangular cartilages and the posterior border of the crus lateralis of the alar cartilages bilaterally as far as the antero-superior angle of the



Fig. 3.

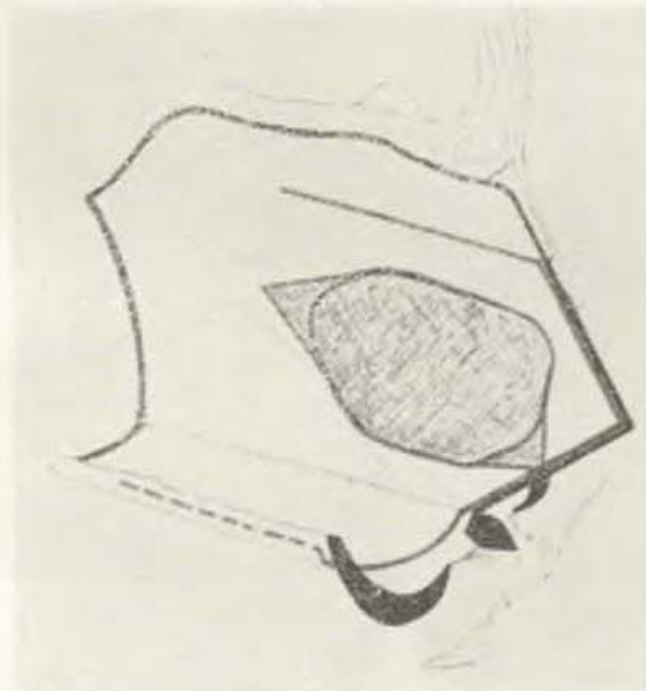


Fig. 4.

quadrangular cartilage; a further retrocollumelar transfixion allows the liberation of the septum base after sectioning the base by a V-shaped incision.

*Stage 2: Removal of the soft parts which are turned upwards. [Fig. 2.]*

For this manoeuvre the use of a lancet is advantageous: damage to the tissue will consequently be minimal. The nostrils and the base of collumela, after having been turned upwards, are fixated by fine silk sutures to the cloth at the rim of the operation field: in this way the field is cleared and rid of retractors and hooks which could hinder the individual surgical manoeuvres.

*Stage 3: Medial section and lateral dislocation of the triangular cartilages. [Fig. 3.]*

The angle between the lateral cartilages and the septum is then accurately sectioned with small, but strong scissors in such a way as to leave the lateral cartilages adherent only to the inferior border of the ossa nasalia; the next section may be already carried out with a very fine scalpel, and it may reach also the nasal bones along the same medial double-wall if a better visibility



of the nasal cavities is required, or if it is necessary to carry out a further surgical stage with the resection of the ascending maxillary branches.

It is also necessary to divaricate the triangular cartilages and fasten them to the cloth at the edge of the operation field by means of silk sutures.

*Stage 4: Sectioning and successive removal of the mucosa of the nasal cavities. (Fig. 4.)*

This stage consists of:

1. Two bilateral symmetrical incisions of the fibromucosa of the septum, starting at the inferior border of the osseous arc and directed posteriorly in a course parallel with the nasal floor and ending about 1 cm behind the posterior margin of the perforation.

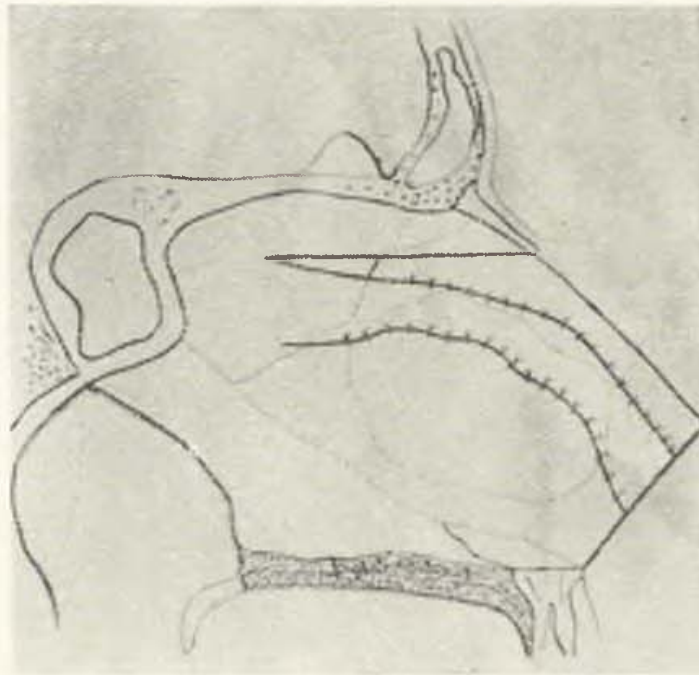


Fig. 5.

2. Two bilateral, symmetrical incisions starting at the inferior interseptocolumellar end and leading parallelly with the interior vestibular orifices to the floor of fossae nasales. After reaching the level of the inferior passages they turn back along the angles of the double-wall of the individual nasal passages and end approximately 1 cm behind the orifice of the choanes.

It is recommended to start removal of the mucosa at the level of the osseous margin of vomer where mucoperichondrium is attached to the mucoperiosteum.

This, in fact, is the very place where uncalled for lacerations of the mucosa may occur which could jeopardize the good result of the intervention.

*Stage 5: Mobilisation of the mucosa and secondary suturing in an adequate position. (Fig. 5.)*

After mucosa is peeled off and edges of the perforation are freshened the borders of both mucous layers — the upper and lower one — are approached to each other from both sides at the level of the perforation and fixed by



a suture in the middle. The suturing is then finished with finest atraumatic zero-six chromium-catgut sutures after having previously removed the superfluous mucosa from both mucous triangles which form inevitably at the end of the suture. Thus reconstructed mucous layers are placed again on the nasal septum and care must be taken that the individual suture lines of the perforation do not overlap which is essential in preventing possible relapses.

The reconstruction is finished by another suture which has to fixate the free rim of each flap over the respective of the inferior flaps are therefore not necessary.

*Stage 6: Eventual correction of the deviated septum and aesthetic modelation of the nose.*

After reconstruction of the nasal septum is finished modelation of the pyramid can be carried out, when necessary, in cases of cosmetic defects by methods usual in otorhinoplastic repairs: deviation of the superior cartilaginous arc may be frequently corrected with success using the triangular cartilages, eventually the posterior half of crus laterales of alar cartilages which may be sutured in an adequate position.

*Stage 7: Reposition and suture of the pyramid base.*

After having finished the plastic reconstruction the nasal pyramid is repositioned and sutured to the face. It is preferable to use the same chromium-catgut and to proceed as follows: an angle suture is placed into the tip of the V-shaped section at the base of columella, then further sutures are layed first at the level of the anterior vestibular orifices and then along the incision line along sulcus alaris. At the end the interseptocolumellar suture is performed by a transfixion with a straight needle.

The intervention is completed by the application of an antibiotic ointment; tamponade of the cavity is not recommended. This is important as a safeguard against even slight pressure damage to the mucosa which could endanger its vitality and also prevent the outflow of mucous secretion from the nasal cavities which could cause secondary infections.

The described procedure, though calling for a very accurate execution, presents no such risks as other methods proposed until now and, compared with them, it offers the advantage of a possible reconstruction in cases of extensive perforations of the septum besides allowing, at the same time, a correction of eventual concomitant aesthetic defects of the pyramid.

#### SUMMARY

After some preliminary informations on the symptomatology of nasal septum perforations, the author records all methods of plastic reconstructions proposed until now which proved to be not only extremely complicated but, moreover, inadequate for repair of extensive perforations. The author describes his technique which differs from the previous ones by being less complicated and having the advantage that besides repairing extensive perforations it allows, at the same time, the correction of eventual deformations of the pyramid.

## R É S U M É

### **Une intervention de la plastie reconstructive chez les grandes perforations septales. Une technique personnelle**

A. Marino

L'auteur présente quelques unes des symptomatologies des perforations septales tout en soulignant le fait que toutes les méthodes de la reconstruction à l'aide de la plastie jusqu'alors pratiquées se sont montrées non pas seulement comme extrêmement compliquées mais en surplus insuffisantes quand à la réparation des grandes perforations.

L'auteur décrit sa propre méthode laquelle — toute différente des autres — n'est pas seulement compliquée comme celle-ci mais, au contraire, elle présente un avantage très favorable quand à la possibilité de réparer les grandes perforations, permet en même temps la correction des déformations éventuelles de la pyramide.

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

### **Plastisch-rekonstruktiver Eingriff bei grossen Perforationen des Nasenseptums. Eigene Technik**

A. Marino

Nach der Anführung einiger Angaben über die Symptomatologie der Perforation des Nasenseptums erwähnt der Autor, wie sich alle bis zum heutigen Tag vorgeschlagene Methoden der plastischen Rekonstruktion nicht nur als extrem kompliziert, sondern vor allem als inadequat erwiesen haben, um grosse Perforationen reparieren zu können.

Der Autor beschreibt seine eigene Methode, die im Gegensatz zu anderen Verfahren nicht nur unkompliziert ist, sondern auch darin vorteilhaft ist, dass sie — ausser der möglichen Korrektur grosser Perforationen — gleichzeitig eine Korrektur der eventuellen Pyramidenformationen ermöglicht.

## R E S U M E N

### **La intervención plástica de reconstrucción en las perforaciones grandes del tabique de nariz. La técnica personal**

A. Marino

El autor después de indicar algunos datos sobre la sintomatología de la perforación del tabique de nariz, indica los métodos de la reconstrucción plástica hasta hoy propuestos, que manifestaron no solamente como extremadamente complicados, pero sobre todo inadecuados para poder reparar las perforaciones grandes.

El autor describe su método, que al contrario de los otros no es complicado, pero tiene la ventaja, que además de la posibilidad de reparación de las grandes perforaciones permite al mismo tiempo la corrección de las deformaciones eventuales de la pirámide.

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## RESECTION OF THE UPPER JAW WITH ELEMENTS OF A PRIMARY PLASTIC

N. M. ALEXANDROV

As together with the removal of malignant tumors also the surrounding healthy tissue has to be removed, localisation of the blastoma in the upper jaw calls for the resection of the whole maxilla within its anatomical borders, and frequently also of the orbital contents, the fossa pterygoidea, and the ethmoid labyrinth. The typical resection of the upper jaw was, as is usually assumed (A. O. Vereshtshinskij) described in 1827 by Gensoul. This operation does not make sure by any means the ablative removal of the tumour; only lately was it completed and extended (Barbosa 1961, Toman et al. 1964), and this no doubt allowed a more radical removal of the tumour. The extension of the amount of interference was made possible only by the introduction into surgery of endotracheal anesthesia lowering thus sharply the number of complications, which it was difficult to combat with before.

Together with the increase in the amount of interference we are faced with the necessity immediately on the surgical table to accept an elementary and usually one-stage plastic operation. This decreases to a considerable extent the mutilation of the patient, improves the postoperative period, and prepares the best conditions for a prosthesis. Otherwise very bad functional and cosmetic deformities must be expected.

The primary plastic operation has been dealt with already previously (P. P. Diakonov 1907, V. Handrikov 1875, Hildebrand 1906, König 1900, et al.). But even more attention is being paid to it presently (I. I. Jermolajev, S. V. Laniuk, R. M. Naumov 1965; N. N. Sokolov 1939; Pollack 1957; Toman 1964; and others).

Tab. 1. Age of patients with malignant tumours of the maxilla.

Before 10 years	11—20 years	21—30 years	31—40 years	41—50 years	51—60 years	61—70 years	71—80 years
3 (2.4 %)	5 (4.0 %)	21 (16.8 %)	18 (14.4 %)	26 (20.8 %)	30 (24.0 %)	20 (16.0 %)	2 (1.6 %)



125 patients suffering from malignant tumours of the maxilla were admitted between the first of January 1952 and the 31st of December 1966 at the Jaw-Facial Surgical and Stomatological Department of Kirov's Military-Medical Academy. In 2 patients the malignant growth was spreading from the skin of the cheek or nose, in 6 patients the tumour of the maxilla represented metastases of malignant growth arising in other organs. The age of the patients ranged from 2 to 76 years (Table 1).



Fig. 1. The planning of the cut

The majority of our patients were men (88—70.4%) the number of female patients was considerably lower (7—29.6%). Carcinoma was histologically proved in the majority of cases (96—76.6%). We included in this group also 4 patients suffering from a malignant papilloma of the maxilla. Sarcoma was considerably less frequent (20 — 16 %), melanoma (6 — 4.8 %), and other tumours such as embryocytoma, seminoma, malignant adenoma of the thyroid gland (3 — 2,4 %).

Most of our patients were admitted to our department after being treated previously at some other medical institution. Table 2 shows the classification of our patients according to the stage of their disease. 27 of our patients (21.6%) were considered to be inoperable after the examination. They were treated by X-ray at the department of rentgenology and medical radiology of the Military Academy or other medical institutions.

The data presented on Table 2 prove, that the contingent of oncological patients was a particular one (56% were admitted because of relapses of their disease following the treatment at some other medical institution). The most



Fig. 2a. The wound after the resection of the upper jaw is covered by the skin graft. — Fig. 2b. The graft is sutured by catgut to the border of the wound and pressed by the roll of the iodoforme gauze. The roll is fixed by mattress striches led through the entire thickness of the cheek

favourable results of treatment were observed in patients at the first or second stage of the disease, that is 5 patients (4%).

Table 3 presents what treatment the patients received before and after admission to our hospital.

Surgical treatment was performed in 98 patients as a rule in combination with X-ray therapy (either before operation at other medical institutions, or after operation at the department for rentgenology and medical radiology of our Academy). In 79 patients resection of the maxilla was performed, in 5 of them bilaterally, in the others recurring tumours were removed electro-surgically (in 6 patients — extraocular exenteration of the orbita).

Removal of the maxilla is followed by very heavy functional derangements: the functions of chewing, swallowing, talking, and the mutilation of the face interfere with the normal mixing of the patients with other people. Therefore, we are of the opinion, that these operations should be performed only by specialists — jaw-facial surgeons — and simultaneously with obligatory one-stage reconstruction as far as possible. A similar outlook is being accepted lately increasingly (P. V. Naumov 1966, M. V. Mukhin 1961, 1965, Colemann 1958,

Tab. 2. Distribution of patients according to the stage of the disease at the time of admission.

Stage	I	II	III/a	III/b	IV	Relapse	All
No. of patients (in absolute numbers)	1	4	23	2	25	70	125
In %	0.8	3.2	18.4	1.6	20.0	56.0	100

Tab. 3. Types of oncological therapy before and after admission to the department for jaw-facial surgery and stomatology.

Type of therapy	X-ray therapy	Operation	X-ray therapy and operation	Untreated
Before admission	33 (26.4 %)	17 (13.6 %)	20 (16 %)	55 (44 %)
After admission	9 (7.2 %)	54 (42.2 %)	44 (35.2 %)	18 (14.4 %)

Conway, Murray 1953, Joss 1964, Straatsma 1956 et al.). The patient must be in the postoperative period under the control of a surgeon and an orthodontist to allow the construction of a dental, and if indicated also a facial prosthesis at the first possible moment (U. V. Aksenov 1967, I. S. Rubinov 1965, D. A. Entin 1924, Pichler 1923, Werner 1963 et al.).

Beginning from 1963 we decided to make use of the various suggestions of different authors and added new improvements to the basic method of operation. This was achieved firstly, when a complete removal of the tumour seemed to become possible during the operation, secondly, when the skin cover of the cheek had to be included into the block of removed tissue.

The widely known incision — Weber, Dieffenbach, Fergusson, Nelaton, Velpeau et al. — (ref. after Tillmans G. 1913) seemed to be of little value as

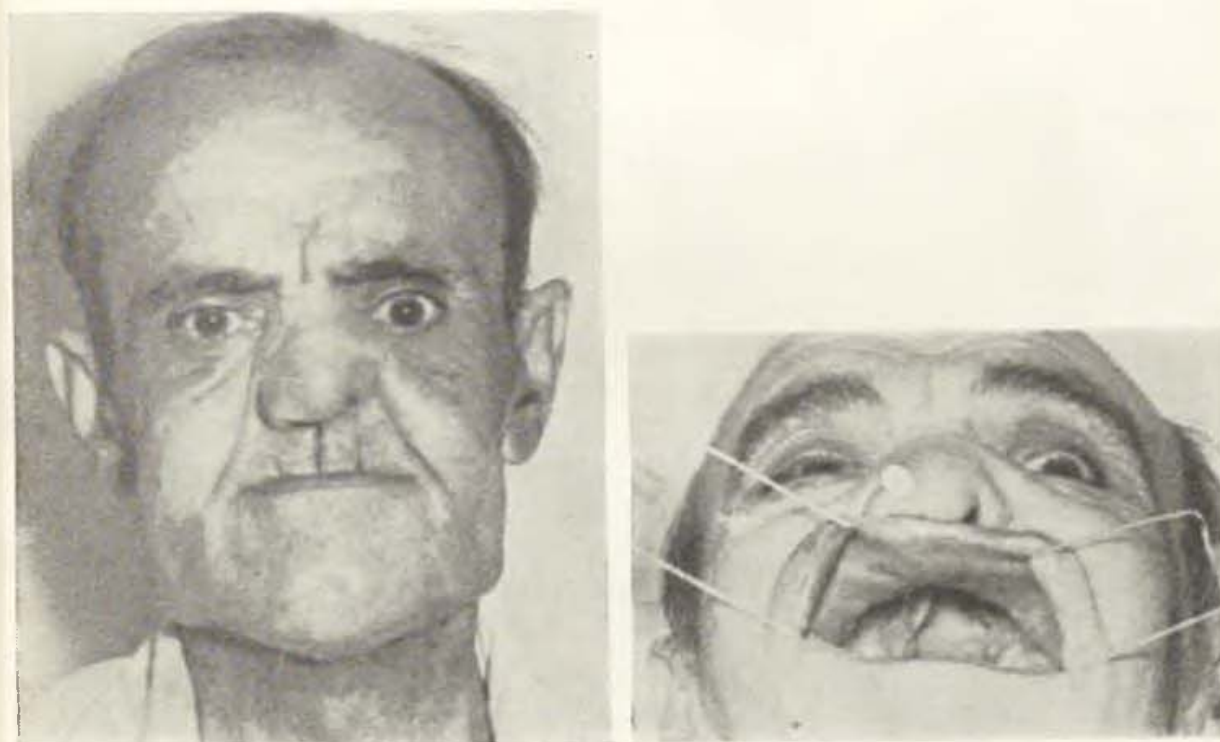


Fig. 3a. Patient C, 3½ years after bilateral resection of the upper jaw. — Fig. 3b. The opening of the mouth is free and the skin graft healed in perfectly



they lead to constant uncorrectible mutilation of the face. We consider the cut according to Kuster (1906) to be very suitable giving an excellent cosmetic result. This cut (Fig. 1) is performed through the middle of the upper lip, goes round the basis and the wing of the nose towards the localisation of the growth, then rises to the inner angle of the eye, turns in a lateral direction, and goes along the edge of the lower lid (we recommend to lead the cut downwards 1—2 mm). The cut runs as far as the outer edge of the orbita, from where it can be prolonged if necessary into the direction of the tragus.

We perform the resection of the bony junctions of the upper jaw using Liston's osteotome and forceps only. Gigli's saw is not suitable for this purpose, also the danger of dissemination of cancerous cells into the wound is higher. We insist on the separation or intersection of the processus pterygoideus from the jaw by using the osteotome since they may represent an undesirable additional resistance during the luxation. We never perform the luxation of the jaws using bone forceps as they splinter the bone, and in all cases interfere with the ablasticity. This part of the operation is best performed with the hand covering with a napkin the surfaces of the jaw which are within reach. We include into the block of removed tissue the entire contents of the orbita, when indicated, and preserve as far as possible the lids and conjunctival sac.

A huge wound surface, which represents the walls of the formed cavity arises after the removal of the jaw. The tamponade of this cavity with iodoform impregnated or simple gauze does not prevent intoxication during the post-operative period. This can be explained by the resorption of products of de-



Fig. 4a. Patient K. V., 4 years and 2 months after the resection of the right upper jaw. —  
Fig. 4b. The defect in the palate



struction through the tissues deprived of epithelium. In the further course the scar formation and epithelisation proceed slowly through many months, and lead to even worse mutilation of the face, and for a long time interfere with the construction of an adequate constant dental prosthesis without which the patients cannot talk or eat.

Pichler in 1923 suggested that a free skin transplantation on the wound should be performed immediately after the resection of the upper jaw. This method was used in 1939 by N. N. Sokolov but unfortunately abandoned in later years. We have been using this method in 26 patients. The method of operation is very simple. A thin skin graft is removed from the thigh towards the end of the operation using a dermatome (cca 10X5 cm). This skin graft is fixed by catgut sutures to the ends of the wound (N. M. Alexandrov, I. P. Golyan 1966). This is performed so that it leaves at the wound edges a stripe for the ingrowing of the cheek graft in its place (Fig. 2a). Further the graft is pressed to the wound by iodoform tampons, which are suitably sawn to the graft by matrace stitches leading through the entire thickness of the cheek (20). Then follows a normal tamponade and the construction of a protective temporary dental prosthesis (or a plate). The first dressing is performed on the 7—8th day, and on the 5th day, when there is a strong oral smell.

König in 1900 used the temporal muscle to prevent the lowering of the eyeball after the removal of the orbital floor together with the maxilla. He resected the coronal process of the mandibula, and after turning the temporal muscle to the floor of the orbita he sew it to the remains of the frontal process of the maxilla. We used this method in 21 patients. In order to prolong the muscle and for the purpose of denervation we always severed the strong tendon leading to the top of the coronal process. The fixing to the frontal process can be carried out with a polyamide thread or simply with catgut. We also use this method, if the contents of the orbita had to be removed, — the muscle fills out well its fornix, and by lowering the drop of the lids creates suitable conditions for an eye prosthesis. With a muscle plastic operation a free skin graft is put on the muscle.

In order to prevent postoperative scar contractures it is advisable to remove not only the coronal process with the temporal muscle and to carry out immediate epithelisation of the wound but also to sever the inner pterygoid muscle.

The use of these above described measures of a primary plastic operation makes the postoperative period incomparably easier. The day after the operation the majority of the patients leaves the bed, and in the third week a constant dental prosthesis-obturator is constructed. The presented photographic pictures (Fig. 3, 4, 5) show, that the face of the patient inspite of the performed extensive operation is not mutilated, the mouth opens freely, the produced bed for the prosthesis insures well the function of a dental prosthesis, and the patients can not only return to their previous surroundings but even to work.

We cannot judge the remote results of the treatment as only four years passed since the introduction of this method. The early results have been

lately incomparably improved, but this can be explained more than anything not by the primary plastic, but by the improvement in surgical technique, by better postoperative X-ray therapy, by improvement in anesthesia, which enables us to work under good conditions, without superfluous haste. One thing is certain — primary plastic operation has no bad influence on prognosis, while it provides numerous very valuable advantages.

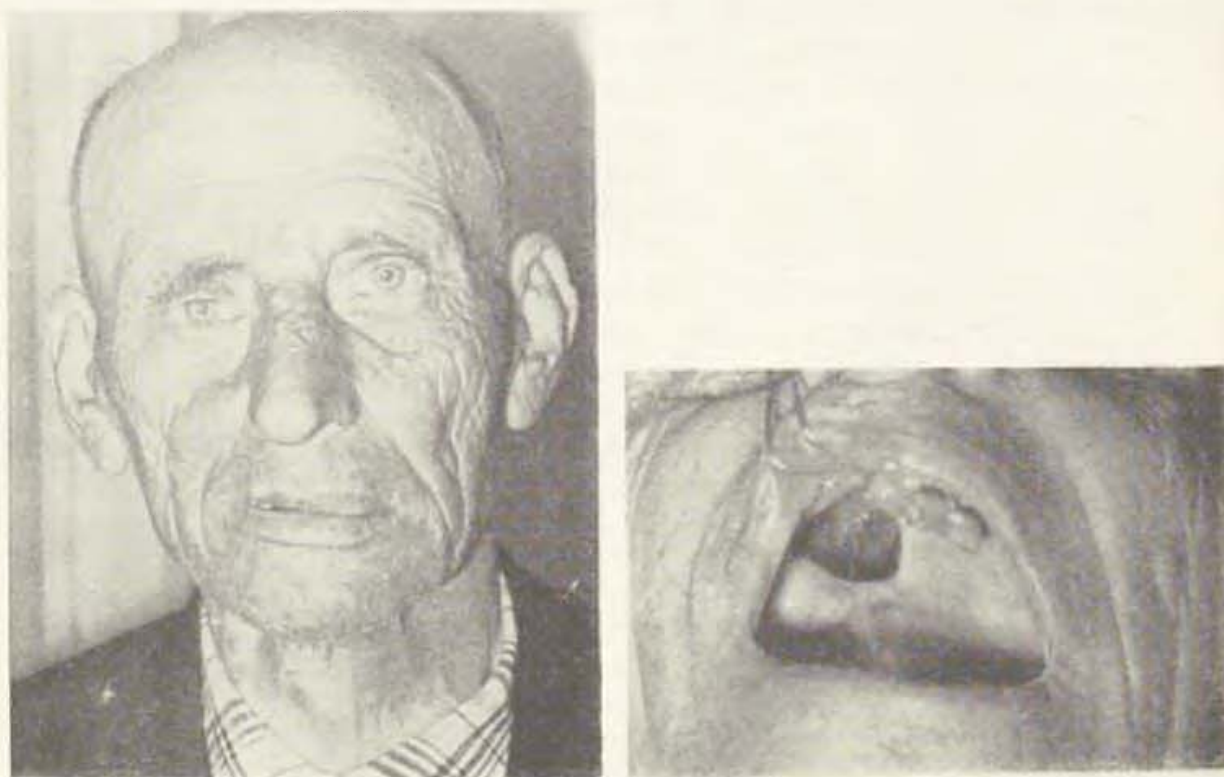


Fig. 5a. Patient 4 years after the resection of the right upper jaw. — Fig. 5b. The recovered skin graft can be seen through the defect in the palate

Preoperative X-ray therapy in our patients was usually performed long before operation, and it had no influence on the healing of the wound and the ingrowing of the skin grafts. The same applies to postoperative X-ray therapy, which we started 8—12 days after operation. The intraarterial (and even more the intravenous) application of cytostatics had a marked influence on the process of regeneration.

We have yet to mention specially Largie's method (1853) (cited from V. A. Ratimov 1882), which envisages the preservation of a mucoso-periosteal palatal graft. This is then sown to the mucosal covering of the cheek, thus dividing completely the oral from the nasal cavity. This provides very good conditions for a prothesis, but on the other hand it strongly worsens the visibility of the cavital walls, and in the case of a relapse of the tumour it can be easily overlooked. We applied this method in 4 patients. In one of them a relapse occurred later, which was not noticed in time, because of bad visibility of the wound. Therefore, we are of the opinion, that this method should be used only in very limited indications. It can be used after the removal of a benign growth, or in

those cases, where the tumour is situated superficially close to the skin, and the skin covering had to be removed together with the jaw and does not interfere with the visibility of the wound.

### CONCLUSIONS

1. When planning operation of malignant growth in the jaw-facial area we have to consider not only the ablasic removal of the tumour, but also steps towards the liquidation of the postoperative mutilation.

2. Primary plastic operation, although it somewhat prolongs the procedure, considerably improves the postoperative period, and allows the patient to return earlier to his accustomed surroundings and to work.

3. If we succeed to carry out the resection of the maxilla ablastically, and to preserve a skin-muscle cheek graft, it is always advisable to end up the operation with a muscle plastic after König, and a free skin transplantation after Pichler-N. N. Sokolov.

4. The severing of the temporal and inner pterygoid muscle appears to be a prophylactic procedure against postoperative muscle contractures, and it has always to be performed.

5. Largie's method can be recommended only after the removal of benign tumours or in those cases, where the block of removed tissue includes also the skin covering the cheek, and the alveolar and palatal processus clinically appear to be not affected.

### SUMMARY

The removal of malignant tumours of the upper jaw results in a lasting interference in the function of swallowing, chewing, and talking. The author is of the opinion, that simultaneously with the removal of the tumour a complex of simple plastic adaptations must be performed in order to improve the state of the patients in the postoperative period, and to enable them to return to work early. It is recommended to carry out a one-phase muscle plastic operation after König, a free skin transplantation after Pichler - N.N. Sokolov, and to sever the upper and inner pterygoid muscle.

### RÉSUMÉ

#### **La résection de la maxille à l'aide de la plastie primaire**

N. M. Alexandrov

La résection des tumeurs malignes de la mâchoire supérieure résulte dans des endommagements de la fonction quand au sens d'avaler, de mâcher de même que de parler. L'auteur présente l'opinion que, simultanément avec la résection du tumeur, il faut réaliser tout un complexe de simple opérations plastiques pour améliorer l'état du malade dans la période même postopérative, servant à rendre le malade le plus tôt que possible dans son travail ordinaire. C'est pourquoi l'auteur recommande de réaliser dans une session l'opération plastique musculaire de König, le transplant cutané libre d'après Pichler - N. N. Sokolov de même que la suture de muscle ptérygoidien externe et interne.



**Resektion des Oberkiefers mit gleichzeitiger primärer Plastik**

N. M. Alexandrov

Die Entfernung eines malignen Tumors des Oberkiefers beeinträchtigt dauernd das Schlucken, Kauen und Sprechen. Der Verfasser ist der Ansicht, dass gleichzeitig mit der Entfernung der Geschwulst ein Komplex von einfachen plastischen Adaptationen anzuwenden ist, um den Zustand des Patienten im postoperativen Zeitabschnitt zu bessern und ihm so eine frühzeitige Rückkehr zur Arbeit zu ermöglichen. Es wird empfohlen, eine Ein-Phasen-Muskel-Plastik nach König vorzunehmen sowie eine freie Hauttransplantation nach Pichler - N. N. Sokolow und den oberen vom inneren M. pterygoideus zu separieren.

## RESUMEN

**Resección de la mandíbula superior con los elementos de plástica primaria**

N. M. Alexandrov

Remoción de tumores malignos de la mandíbula superior causa defectos permanentes de la función de deglucir, de masticar y de hablar. En la opinión del autor es necesario al mismo tiempo con la remoción del tumor realizar un complejo de sencillas adaptaciones plásticas para mejorar las condiciones de los pacientes en el período postoperatorio y para posibilitarles pronto regreso al trabajo. Se recomienda a practicar la operación plástica muscular de una sola fase según König, la transplatación de la piel libre según Pichler - N. N. Sokolov y separar el superior e interior músculo pterigoideo.

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## A CONTRIBUTION TO THE DEGRADATION OF SKIN AND CORNEA PRESERVED HEMIBIOTICALLY IN THE TEMPERATURE NEAR 0° C (Brief communication)

R. KLEN, J. HEGER

Cold preservation of tissue grafts is a simple and often a suitable method if the tissues are stored for a short period. Low temperature, in the first place, retards autolysis and, in the second place, hampers the activity of the mesophilic microbes which are usually the main cause of putrefaction. The ability of some bacteria to grow at a temperature about 0 °C, but even at lower temperatures, has been known since 1887 [1]. These microbes are referred to as psychrophilic microbes although this term does not adequately convey their properties. In the present work, we have classified psychrophilic microbes into the obligatory ones (those which grow better at +4 °C than at +37 °C), and the facultative ones (which grow equally well at +4 °C or +37 °C). This classification corresponds to a model for in some strains, psychrophilia can be developed through adaptation [2]. From the point of cold preservation of tissue no attention whatever has been given to psychrophils so far, although their importance for transfusion service is well known [3, 4]; and there exists an ample literature dealing with their occurrence and action on cold preserved food [5]. Since the psychrophils are omnipresent in the external environment of man and since the skin and the cornea are in immediate contact with them, we must take it for granted that they are present on these tissues. If we preserve these tissues hemibiotically we cannot successfully sterilize them and we therefore cannot declare them to be sterile only on the basis of a negative culture of mesophilic microorganisms. The finding of psychrophils on cold preserved tissues is of importance both for the assessment of advancement of disintegration of the tissue and for the interpretation of some reactions shown by the recipient.

Since in this communication we are going to deal only with the interpretation of our basic findings of psychrophils on the skin, on the cornea and in the conjunctival sack [6, 7], we only report on the methodical data which have not been dealt with as yet.

## METHODS

Thiersch grafts were taken by means of Humby's knife from the ventral surface of thighs of cadavers corresponding to indications of skin withdrawal for clinical practice (8). In the first 16 donors uncleansed skin was taken from the one thigh while the skin from the other thigh was three times thoroughly cleansed with ether. The number of examinations of the cleansed skin was completed to 50. Each graft was cut in sterile manner into 10 portions sized about 0.5 cm<sup>2</sup> each. Five of them were cultivated immediately after cutting, the rest after 3-week's storage in a sterile wet chamber at +4 °C. That donor was considered positive in whom microbes were obtained by culture from at least one portion of the graft. We have found 6 obligatory psychrophils and classified them according to their families; their identification as to species, as far as it has been possible, is not quite reliable. Further on, we have found 7 facultative psychrophils, out of which 4 have not been identified at all because they got lost immediately after they had been cultured. Simultaneously, we carried out an orientative examination of mesophils. On examining 160 corneae we obtained smears from the cornea and the conjunctival sack by means of a swab. Fifty corneae were examined in living subjects with a normal ophthalmologic findings. Another 110 examinations were carried out on cadavers. Out of them examinations within 2 hours after death were carried out on 50 eyeballs, in the period between 2 to 20 hours on 40 eyeballs, and in the period between 20 to 48 hours on 20 eyeballs. The cadavers from which the smears were taken within 2 hours after death fulfilled the conditions of cornea withdrawal for keratoplasty (8).

## RESULTS

In all of the 16 donors of non-cleansed skin mesophils were found before preservation; in 6 cases there were obligatory psychrophils and in 4 cases facultative psychrophils. On the cleansed skin mesophils were also found before preservation in all of the same 16 donors; in 6 of them there were 6 obligatory psychrophils and in 5 facultative psychrophils. In 50 donors of the cleansed skin mesophils were found before preservation in all cases. In 9 cases obligatory psychrophils, in 11 cases facultative psychrophils were present. After preservation mesophils were observed in 15 cases, obligatory psychrophils in 9 cases and facultative psychrophils in 7 cases in the 16 donors of non-cleansed skin. In the same 16 donors of cleansed skin, mesophils were found in all cases, obligatory psychrophils in 5 cases and facultative psychrophils in 4 cases. In 50 donors of cleansed skin, mesophils were present in 48 cases, obligatory psychrophils in 9 cases, and facultative psychrophils in 11 cases. Since the method used does not allow to culture the same sample prior to and after preservation, the positive results obtained in one and the same donors prior to preservation and after it were not always identical. For this reason we have calculated, the total number of positive donors which expresses the sum of contaminated donors in a better way. Mesophils were always present on non-cleansed as well as on cleansed skin. Obligatory psychrophils were present

In 12 cases on non-cleansed skin; in 7 cases on cleansed skin of the 16 donors; in the 50 donors of cleansed skin they were present in 15 cases. Facultative psychrophils were found on non-cleansed skin in 8 cases, in 6 cases on cleansed skin of the 16 donors; with the 50 donors of cleansed skin they were found in 17 cases. Out of the total number of 15 obligatory and 17 facultative psychrophils observed in 50 donors of cleansed skin, in 8 cases a positive finding was observed only either before the preservation so after it. The total number of donors with psychrophilic microbes amounted thus to 24; out of these cases, only obligatory psychrophils were present in 18 donors, only facultative psychrophils in 2 donors, while both kinds were present in 4 donors.

In thirteen cases we have found contamination with only one family of psychrophils, in 7 cases with two ones, in two cases with three, and in 1 case with four or five respectively. To a certain extent, this finding is indicative of the degree of infection, and in correspondence with another orientative quantitative indicator, namely the finding that the number of positive specimens (out of a total of 5 under study) in those donors where only one family had been established, was smaller than in those donors where several families had been established. Among the obligatory psychrophils the *Micrococcus candidans* was by far the most frequent finding. Among the facultative ones *Escherichia coli* and *Streptococcus faecalis* were the most frequent while their number was the same.

We simultaneously followed up the occurrence of moulds. We are well aware of the fact that their finding would be greater if special methods of culture were used. But even so the obtained results are of importance. In 16 donors of noncleansed skin we observed them, after preservation in 5 cases in the same 16 donors of cleansed skin originating from the other thigh in 4 cases; in 50 donors of cleansed skin we observed them, after preservation, in 20 cases, i.e. 40 %. Among them, in 6 cases they were visible direct on the with-drawn skin when the preservation was finished. They belonged to the genus of *Mucor*, *Rhizopus* and *Penicillium*.

Among the 50 smears from the eyeballs of living subjects we established mesophils in 14 cases, one obligatory psychrophil in one case and one facultative psychrophil also in one case. Among the 50 smears carried out within 2 hours after death mesophils were found in 47 cases, obligatory psychrophils in two cases, facultative psychrophils in 8 cases and *Torula* in one case. Among the 40 smears carried out between 2 and 20 hours after death mesophils were obtained by culture in 39 cases, obligatory psychrophils in 2 cases, facultative psychrophils in 8 cases and mould in one case. Among the 20 smears carried out between 20 and 48 hours after death mesophils were found in all cases, obligatory psychrophils in 5 cases and facultative psychrophils in 11 cases.

The number of positive results in donors up to 2 hours after death was increased by 66 % in mesophils and by 16 % in psychrophils, as compared with living donors. The increase in positive results in the period between 2 and 24 hours made 4 % in mesophils and 2,5 % in psychrophils. In the period between 24 and 48 hours it amounted to 2 % in mesophils (thus reaching 100 %) and



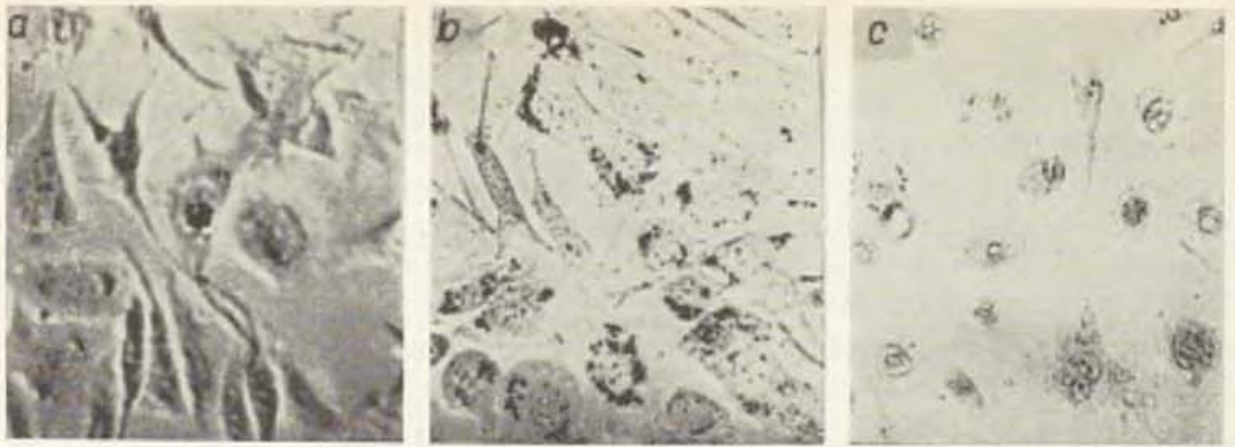


Fig. 1. Mouse L-fibroblasts cultivated 72 hrs in the temperature  $+37^{\circ}\text{C}$ . From left to right: a = control, b = Rifampicin, c = Framycetin 500  $\gamma\text{ml}$

in psychrophils it rapidly raised from 22.5 % to 80 %. The curves of multiplication of microbes are in keeping with the well know about multiplication of mesophils and psychrophils.

From the practical point of view, the occurrence of psychrophils on the cornea and conjunctiva of living subjects and 1 donors up to 2 hours after death may be of importance. In the living donors we observed the obligatory psychrophil *Micrococcus candidans* in one case and the facultative *Escherichia coli* in one case. In the donors up to 2 hours after death we obtained by culture, from the obligatory psychrophils, *Pseudomonas myxogenes* in 2 cases, and *Torula* in one case; *Escherichia coli* in 4 cases and *Pseudomonas spec.* in 4 cases from the facultative ones.

#### DISCUSSION

Since biochemical properties of psychrophils are often atypical, their identification is difficult even in specialized bacteriological laboratories. Our work was thus not aimed at the identification of these microbes but at gaining data about their occurrence on the tissues which cannot be obtained in sterile condition for transplantations and which are preserved hemibiotically. The finding would certainly have increased if a special medium (9) had been used simultaneously with the universal media. Another circumstance which may have played a role was perhaps the season of the year because in the colder period (February to April) the occurrence was more frequent in the than hotter period (May to August). We believe that personal hygiene also played a part in such a way that the occurrence of psychrophils, on the skin was the more frequent, the oftener the donor washed himself with water [which contains up to 47 % of psychrophils out of the total amount of microbes (10)]. It is certain that contamination of the space in which the dead body is harboured before the withdrawal, and its temperature, play a role too. From this point of view, storing of the dead body in a cooling room is a negative moment. The occurrence of psychrophils decreased to some extent when the skin was washed, but it still



remained fairly high. We have deliberately omitted rinsing of the conjunctival sack and of the cornea since on them we expected an essentially lower occurrence than on the skin. This expectation was confirmed. The importance of our study is demonstrated by the fact that psychrophilic microbes have modest demands on nutrient substances, and the facultative ones (which represent approximately one half of our material) have a broader temperature living zone than the mesophiles, thus causing putrefaction even at temperatures at which it is usually not thought of. From this point of view the yeast is still more important for its lower temperature limit lies still deeper and it can multiply even below the freezing point which, however, requires inhibition of psychrophils (5). Our finding of *Torula* also speaks for it.

Although psychrophils are not human pathogens, their presence in the human body is not unimportant. This is well known from transfusion of blood contaminated with them (3, 4), which may have a fatal outcome (11). Brande (12) mentions that toxicity of psychrophilic *Pseudomonas*, coliform microbes and *Achromobacter* is almost identical with the toxicity of current intestinal bacilli. Kaeashima (13) has isolated a strain which produces large quantities of histamin, so that after ingestion of food contaminated with it poisonings reminiscent of allergy resulted. If we now take into account the fact that in subjects in whom transplantation has been performed, the response to non-pathogenic microbes may be changed, it is obvious that examination for psychrophils constitutes an indispensable part of examination for the assessment of suitability for transplantation of tissues preserved hemibiotically during at least 17 days at about 0 °C in the same way as in the assessment of cooled blood for transfusion.

The differences in the occurrence of psychrophils prior to and after preservation can be explained in such a way that on some specimens the microbes were not present at all. After the preservation a greater occurrence must be expected, since the microbes had the possibility of multiplication. Buttinaux and Catsaras (14) have found that psychrophils on the skin slaughtered cattle, stored at low temperature, multiply rapidly. On the first day, they found 300

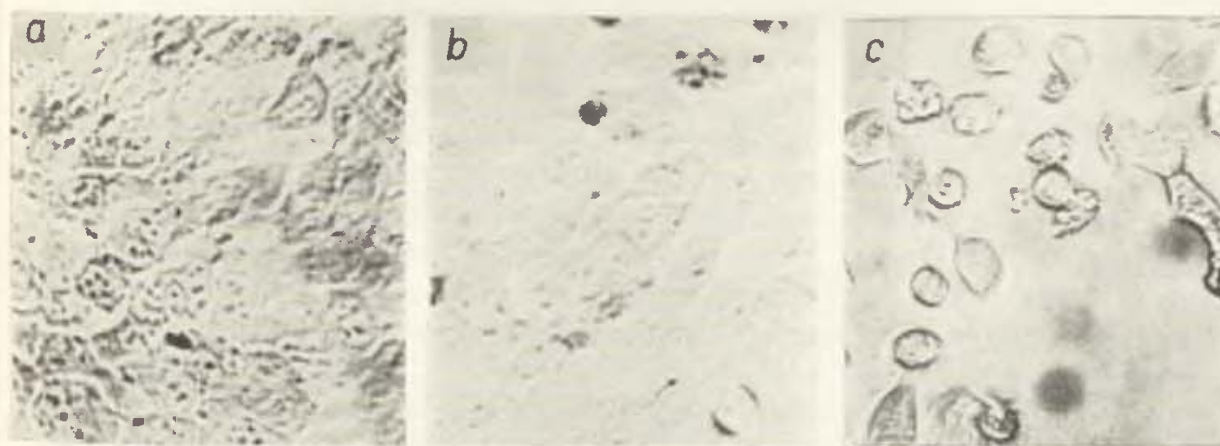


Fig. 2. HEp cells cultivated 48 hrs in the temperature + 37 °C. From left to right: a = control, b = Rifampicin, c = Framycetin 500 γ/ml

microbes on 1 m<sup>2</sup>, after 2 days 1.300, after 4 days 75.000 and after 8 days 40 millions. We ourselves have tested this great ability of multiplication in *Micrococcus candidans* and *percitreus* when we performed massive infection of grafts with them. After 3 week's preservation their growth was macroscopically well visible.

The fact, that also the psychrophilic microbes found by us are aerobic microbes, is of importance from the point of method of preservation, because we can suppress their activity by creating anaerobic conditions. We have already made this experience when studying putrefaction of articular cartilage infected with *Penicillium nigricans*. The growth of this mould at +4 °C was — judging by its parasitic action on the tissue — smaller on the graft preserved under anaerobic conditions, i.e. immersed in paraffin oil — than under microaerobic conditions, i.e. in gauze soaked in F 1/2 solution and in CO<sub>2</sub> atmosphere (15). This is probably one of the factors responsible for the success of tissue preservation in paraffin oil (16).

Our orientative examination of mesophils of the cornea and the conjunctival sack is in keeping with the well known data [e.g. 17, 18], we only performed it as a control of our method. The number of psychrophils found on them is much smaller than that on the skin though it substantially increases post mortem as compared with the count established in living subjects. We see the cause in the tissues of the eye under examination being covered with the eyelids and in the action of the tears which contain bacteriostatic substances.

Among the 20 donors of skin on which moulds were found, antibiotics or and steroids, had been used for treatment. Since 63 % of all our donors have been treated with antibiotics (19) we believe that this factor may play its role during preservation, when the tissue is almost completely deprived of natural defense abilities and becomes only a good nutrient substrate for microorganisms. Also in the case where we observed *Torula* on the cornea, the subject had been treated, among others, with antibiotics. With the exception of the four non-identified facultative psychrophils, i.e. in 92,4 % of the psychrophils was performed in vitro examination for their sensitivity to 21 antibiotics. On the evaluation of the antibiotics, we took as a basis, on the one hand, the lowest concentration at which we could observe a safe inhibition of growth, on the other hand, the occurrence of the microbe. These results were statistically evaluated by computing weighted means which were mutually correlated by the t-test. "Pathogenicity" of the microbes for the grafts could not be taken into account in the evaluation because no accurate data were available in this respect, although it is obvious that these are considerable differences in the quality of the putrefactive effect of *Pseudomonas* and, e.g. *Flavobacterium*. We evaluated the results in sensitivity in vitro as absolute results because we almost completely imitated the conditions under which the process takes its course.

We shall confine ourselves to mentioning the most important data about Rifampicin-Lepetit which proved to be the most suitable. Its only weak point lies in its ineffectiveness on *Escherichia coli* which, however can be compensated by combining it with some other antibiotic, e.g. streptomycine.

This pair of antibiotics make a very good whole extending their inhibitory effect a large spectrum of the psychrophils found by us without interfering with each other. A great advantage of Rifampicin-Lepetit is the great inhibitory effect on *Pseudomonas*, which we consider to be probably the most "pathogenic" for the graft of all the observed microbes, and its acting upon 70 % of the found psychrophils at the least tested concentration of 10 gamma/ml. This is certainly in connection with the well known fact that bacteriostatic action, unlike the bactericidal, increases with decreasing temperature. It is quoted that a  $1/10$  concentration of an antibiotic or of sulphonamides is sufficient to inhibit growth of psychrophils at temperatures of  $+10$  to  $+3$  °C, to distinguish from psychrophils living at normal temperature [20]. Only upon microbes of the species *Alcaligenes* Rifampicin-Lepetit acts at a relatively high concentration of 500 gamma/ml. The presence of these microbes, from the point of healing of the wound, however, will probably have a favourable effect because it is connected with alkaline reaction which accompanies their occurrence.

In the first place, however, we appreciate its low toxicity which we tested by culture in vitro on human L-fibroblasts and epithelial HEp cells. We carried out the tests, on the one hand, with growing cells, and, on the other hand, with cells forming monolayers. We determined the time of development of the cytopathogenic effect in the cells to the medium of which we added 500 gamma/ml of Rifampicin-Lepetit or other antibiotics, e.g. Framycain, which also exerts an important inhibitory effect on the growth of psychrophils, and we compared the condition of the cells with that of cells cultured in the usual way.

In brief, the following can be stated: Since Rifampicin-Lepetit is a highly effective antibiotic against the psychrophils registered by us and since it has a very small toxic effect on growing and adult cells in vitro we consider it as a very suitable agent for hemibiotic preservation of tissue grafts at temperatures about 0 °C. By inhibition of the growth of psychrophilic microbes it prolongs the time of preservation and reduces the possibility of posttransplantation reaction in the recipient, caused by psychrophils transferred with the graft. Owing to its low toxicity, it does not essentially impair the viability of the cells of hemibiotically preserved grafts.

#### SUMMARY

The 6 obligatory and 7 facultative psychrophilic microorganisms were found on the human skin, cornea and conjunctival sac.

The occurrence of these microorganisms on the mentioned tissues, in skin also after hemibiotic preservation in wet chamber at the temperature  $+4$  °C is described. To the same time the occurrence of mesophils, moulds and yeast were orientatively tested.

Three most powerful antibiotics against psychrophils, among 21 tested, were evaluated on their toxicity to mouse L — fibroblasts and HEp cells.

From these examinations the authors consider the Rifampicin-Lepetit is, to the time, the best agent, which helps in the hemibiotic preservation near 0 °C.



## R É S U M É

### **Contribution à la dégradation du transplant cutané et celui de la cornée conservé à l'aide des hémibiotiques dans la température autour de zéro**

R. Klen, J. Heger

Les auteurs ont constaté six des sortes obligatoires (3 sortes des micrococques, *Esch. coli* et *Flavobacterium*) et sept sortes facultatives (*Streptococcus faecalis*, *Micrococcus albus*, *Esch. coli* et quatre des sortes pas identifiées) des microbes psychrophiles se trouvant sur la peau, la cornée et la conjonctive des donneurs de ces tissus, et ils décrivent leur distribution. En même temps ils ont soumis à l'examen d'orientation la présence des mesophiles, des moisissures et des levains.

Chez les psychrophiles obligatoires ils ont in vitro examiné leur sensibilité envers 21 des antibiotiques et 7 des antiseptiques et 6 des collyres. Les résultats avec les antibiotiques ont été examinés à l'égard de la statistique. Chez trois des plus efficaces les auteurs ont examiné leur cytotoxicité quand au L. fibroblastes et au cellules HEP en culture sous +37 °C, grandies et conservées ensuite dans des températures de +37 °C, +22 °C et +4 °C. Celui le plus efficace de tous semble être Rifampicin-Lepetit.

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

### **Beitrag zur Degradation des bei 0 °C hämibiotisch konservierten Haut- und Hornhautpfropfens**

R. Klen, J. Heger

Die Autoren fanden 6 Gattungen obligater (3 Gattungen von *Micrococcus*, *Esch. coli* und *Flavobacterium*) und 7 Gattungen fakultativer (*Streptococcus faecalis*, *Micrococcus albus*, *Esch. coli* und 4 nichtidentifizierte) psychrophiler Mikrobe an der Haut und Hornhaut und Konjunktiva der Spender dieser Gewebe, und beschreiben ihr Vorkommen. Gleichzeitig untersuchten sie orientierend das Vorkommen der Mesophile, Schimmelpilze und Hefen.

Bei den gefundenen obligaten Psychrophilen ermittelten die Autoren in vitro ihre Empfindlichkeit gegenüber 21 Antibiotika, 7 Antiseptika und 6 Augenkollyrien. Die Ergebnisse bei den Antibiotika wurden statistisch ausgewertet. Bei den drei wirksamsten wurden Prüfungen an ihre Zytotoxizität bei L-Fibroblasten und HEP-Zellen (wachsend bei +37 °C, erwachsen und weiter kultiviert bei +37 °C, +22 °C und +4 °C durchgeführt. Als wirksamst erwies sich Rifampicin-Lepetit.

## R E S U M E N

### **La contribución a la degradación de la grieta de piel y la dela córnea conservada del modo hemibiótico en la temperatura alrededor de 0 °C**

R. Klen, J. Heger

Los autores verificaron 6 tipos obligatorios (3 tipos de *Micrococcus*, *Esch. coli* y *Flavobacterium*) y 7 tipos facultativos (*Streptococcus faecalis*, *Micrococcus albus*, *Esch. coli* y 4 no identificados) microbios psicrófilos en la piel y en la córnea y conjuntiva de los donadores de estos tejidos y describen su presencia. Al mismo tiempo siguieron para la orientación la presencia de los mesófilos, de los mohos y de las levaduras.



En los psichrofilos obligatorios encontrados comprobaron in vitro su sensibilidad a 21 antibióticos, 7 antisepticos y 6 collirios oftálmicos. Los resultados en los antibióticos avaloraron estadísticamente. En tres, los más eficientes practicaron las pruebas a su citotoxicidad en L-fibroblastos y HEp células crecientes en +36 °C que crecen y después se cultivan en +37 °C, +22 °C y +4 °C. El más eficiente parece Rifampicin-Lepetit.

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## ASSESSING THE BIOLOGICAL ACTIVITY OF CONSERVED TENDON BY TISSUE CULTURE

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Among the problems linked up with homotransplantation, assessment of the qualities of tissues as they change by conservation, is one of the important questions. It will make it possible, to a certain degree, to detect many regular features and thus to determine the optimal conditions and time for conservation and storage of viable tissue.

Although it may be admitted that transplantation of certain dead tissue is rational for mechanical purposes [Billingham, 1956; Klen, 1962], many other authors, on the other hand, rightly point out that viable transplants have many advantages over dead tissue. Klen's theory of transplant viability is based on the modern conceptions of preserving the biological activity of cell elements in conserved tissue. In accord with it, a graft is to be regarded viable, if its metabolism has not suffered irreversible changes (as compared with the metabolism of the same tissue in situ of a normal organism) and if it is capable of growth and regeneration under favourable conditions.

The biological activity of conserved tissue can be defined by histology, vital staining, histochemistry, measurement of tissue respiration, tissue culture and by transplantation.

Reports in the literature as well as our own experience have shown that the results of tissue culture in vitro are objective and very reliable criteria of cell viability.

In recent years, therefore, tissue explantation has, not by accident, been widely employed for determining the biological activity of conserved tissue. Skin was thus tested by Sushko in 1961, Livshits et Chalaya in 1963, Seslavina, Danilova et Fedotenkov in 1965, Timofeyeva et Pimkina in 1966 and Billingham et Medawar in 1952, bone marrow by Fedotenkov, Danilova, Mefedova et Dish-

kant in 1961, Fedorov in 1966, Rakhmatullayev in 1966 and Perry in 1963, cartilage by Kovalenko et Yemelianov in 1966, blood vessels by Bilenko in 1962, cornea by Filatov et Bazhenova in 1936, peripheral nerve by Yatsenko in 1964 and other tissues and cells (Scherer et Hoogasian, 1954; Parkes, 1957; Stulberg, Soule et Berman, 1958; Veen et van Der Mes, 1958; etc.).

We regret not to have found in the literature any study dealing with the biological activity of conserved tendon. The number of articles by Olivo et Galliani, published in 1962, concerned with the mitotic activity of fibroblasts explanted from tendons of chicken embryos and cultivated in vitro after cooling, represent but a side line of the problem investigated by these authors.

Neither in the Soviet literature are there any reports on similar studies, apart from our paper read at the Fifth Congress of Surgeons from the North Caucasus and Rostov-on-Don in Sept., 1963, and published in the procès-verbal of that Congress (Kovalenko, Fedorov, Demichev, Yemelianov, Mikaelyan et Didenko).

Since 1963, we have carried out a number of systematic experiments with tissue culture of tendon which were mainly aimed at the determination of tissue viability after cooling, freezing and lyophilization. These investigations were run along two lines: 1) study of the influence of low temperatures on the biological activity of tendon and 2) study of the part glycerin plays as protecting agent of tendon tissue against low temperatures.

For these reasons, five series of experiments were carried out.

In the first series, small pieces of tendon stripped of paratenon and periten-dineum were cultivated after they had been stored in a glucose-citrate-penicillin solution (according to Kovalenko) at  $+4^{\circ}\text{C}$ . In the second series, tendon specimens were explanted which had been frozen at  $-25^{\circ}\text{C}$  without any preceding treatment and in the third series, these specimens were placed in a 15 % glycerin solution and then frozen at  $-25^{\circ}\text{C}$  prior to explantation. In the fourth series, specimens of tendon were first soaked in 15 % glycerin, then frozen at  $-183^{\circ}\text{C}$  and stored at  $-25^{\circ}\text{C}$  for one to 90 days prior to explantation. In the fifth series of experiments, specimens of tendon were cultivated after lyophilization. Thus a total of 78 experiments with 754 explants were carried out.

In all instances, before explantation, the conserved specimens were warmed up in water of  $+38^{\circ}\text{C}$  for 90 sec. Cultivation was carried out in Carrel flasks, each with five to seven specimens measuring 0.5 to 2.0 mm. and fixed each on a separate glass slide with a drop of cock plasma. After coagulation of the plasma, 2 to 3 ml. of nutritive fluid containing nine parts 199 synthetic medium and one part homogenous serum, were instilled into each flask.

During cultivation, the nutritive medium was exchanged every five to six days. The tendon specimens were histologically examined both in native and stained sections, the latter after fixation in 10 % neutral formalin and stained with haemotoxylin-eosin. Specimens were thus examined after two to ten days cultivation.

#### A. FIRST SERIES

{tendon conserved at  $+4^{\circ}\text{C}$  in Kovalenko's glucose-citrate-penicillin fluid}

Altogether 18 experiments with 178 explants were carried out.

The task was to ascertain how long tendon tissue survived in Kovalenko fluid.

Most of these explants showed active growth in tissue culture which became manifest by single, radially orientated cells at the margin of the explant, but their number grew quickly, so that they soon encircled the entire circumference of the specimen. Agglomeration of these cells around the explant thus formed a radiant halo of a broad productive zone.

After one day of cooling the explants showed good and regular growth in tissue culture. The period of latency, i.e. between explantation and start of cell migration, lasted 36 hours.

After three days conservation the specimens showed the same intensity of growth, and after three days culture the area of new-grown fibroblasts greatly exceeded that of the original explant. At the basis, the cells were more crowded than in the periphery, and by union of their cytoplasmatic projections they formed a dense network.

The period of latency in tendon conserved for five days was much longer; it lasted 48 to 72 hours. However, the zone of cell proliferation was as broad as in the previous experiments. The fibroblasts were orientated radially to the centre of the explant, their outlines were clear and their nuclei well visible.

After nine days of conservation, the period of latency became longer still. The tissue culture originating from the explant presented a not very broad, but rather compact layer of new-grown cells (fig. 1). In some explants, cells resembling fibroblasts, formed foci. Sometimes free cells were found both in the periphery and the proliferative zone. Cells in the stage of mitotic division were met with but rarely.

After eighteen days of conservation and cultivation, growing fibroblasts were found in 15 out of 34 explants. The period of latency increased to 96, even 120 hours. There was little growth of cells and amitoses were met with frequently.

In tendon explants, which had been stored at  $+4^{\circ}\text{C}$  in glucose-citrate-penicillin fluid for twenty-one days growth of cells was irregular and only found in two out of 23 specimens. Mitotic division could be detected, but the cells in the periphery rapidly degenerated. Some distance away from the explant, there were free cells, most of them showing vacuolation.

#### B. SECOND SERIES

{tendon frozen at  $-25^{\circ}\text{C}$ }

The specimens of this series were frozen at  $-25^{\circ}\text{C}$  and, after storage, quickly warmed up in water of  $+38^{\circ}\text{C}$  for 90 sec.

We carried out nine cultivations in a total of 98 explants.



In 96 out of 98 specimens no growth or migration of cells resembling fibroblasts could be found. In the two explants, which had been stored for three and 49 days, only slight growth could be detected.

Before the appearance of the first migrating cells, there was a considerably prolonged period of latency (96 to 120 hours). The cells of the proliferative zone were flat, elongated in the periphery and with nuclei of irregular shape (fig. 2). On the sixth day of cultivation degenerative changes quickly set in; the cytoplasm became darker, the nuclei started to lose their regular outlines and structure and began to shrink, vacuoles appeared and division of cells proceeded by amitosis.

Tendon explants frozen without a protecting medium, therefore, manifested an extremely low viability of cells.

Our results correspond with the well known observations of Rey (1962) who, but in very rare instances, found two to three migrating cells of characteristic spindle shape in heart tissue explants cultivated after freezing at  $-196^{\circ}\text{C}$ , and also with the findings of Smith (1963).

### C. THIRD AND FOURTH SERIES

[tendon treated with 15 % glycerin and frozen at  $-25^{\circ}\text{C}$  (third series) or  $-183^{\circ}\text{C}$  (4th series)]

The discovery of Rostand (1946) and Polge, Smith et Parkes (1949) of the protecting properties of glycerin led to remarkable success with the application of low temperatures in biology.

The conservative properties of glycerin under these circumstances are based on its specific capacity of protecting cells from frost damage (Lovelock, 1953; Lovelock et Polge, 1954) by changing the character of crystallization (Smith, Polge et Smiles, 1951). It is also possible that the increased resistance of cells against frost damage not only depends on the anti-freeze properties of glycerin, but also on its non-specific capacity of increasing the general physiological resistance of tissue against injury (Volfenzon, 1963).

According to Rey (1962), tissue soaked in glycerin acquires, at low temperatures, physico-chemical properties which are characteristic of glycerin and, therefore, react to cooling accordingly.

At present, apart from glycerin in 2, 5, 10, 15 and 50 % solutions (Sushko, 1961; Kovalenko, 1962; Radolitskaya, 1963; Fedorov, 1964; Perry, 1963; Meryman, 1963), a 10 % solution of dimethylsulphoxide (Sushko et Meyerson, 1965; Meryman, 1963), polyvinylpyrolides (Lavrik, Afanaseyeva, Glukhenkaya, Yatsenko et Karabanova, 1966), vaselin oil (Timofeyeva et Pimkina, 1966) and other substances are used for protection against low temperatures.

Based on the results hitherto published, many authors (Rudykh, Yatsenko et Keysevich, 1966) have come to the conclusion that each type of tissue requires a different method of low-temperature conservation.

The following study was aimed at determination of the biological activity of tendon tissue soaked in a 15 % glycerin solution for 30 to 60 min, and then frozen at  $-25^{\circ}\text{C}$  or  $-183^{\circ}\text{C}$ .

Altogether 19 experiments (of the third series) with cultivation of 204 explants and 18 experiments (of the fourth series) with 187 explants, were carried out. The results were analogous in both series, so that it may suffice to describe the findings in one series only.

Explants which had been frozen and stored for one day, showed active growth in tissue culture, whereby no change in appearance or attitude of the specimens could be detected after 24 hours. Cell migration only started after 36 to 48 hours cultivation. At that time, thin, transparent, sharp pointed cytoplasmatic projections of the spindle-shaped or asteroid cells could clearly be seen penetrating the nutritive plasm from the periphery of the explant.

After three to four days of cultivation the explants were surrounded by a dense layer of radially orientated cells of extraordinarily varied shape. There were branched, cylindrical, spindle-shaped and asteroid cells with lanceolate or thread-like projections forming syncytial bridges with each other. The cytoplasm of these cells was transparent and clear, the cells of regular, oval or round shape with a thin and pale cell membrane and with two or three large and irregularly shaped nucleoli. Sometimes, a few free or, as Maksimov (1916) called them "roaming" cells could be found. They usually were scattered and single or formed small clusters among the projections or spindle-shaped cells (fig. 3). Their sharply outlined, dark cytoplasm contained small granulae looking like tiny angular lumps. The nuclei were brightly stained and had an irregularly plicate appearance. In the migrating spindle-shaped cells and the free cells, the process of mitotic cell division could well be followed up. During mitosis, the cells became bigger, more rounded and their projections disappeared.

In a four- or five-day culture, the explants were encircled by a broad belt of new-grown tissue, very compact around the margin of the explant and getting thinner toward the periphery, but everywhere with the characteristic radiate arrangement of cells.

The migrating cells had preserved their typical shape of thin spindles or asteriods with branche-like and cylindrical projections. A few cells, particularly in the periphery, were quite isolated, but most of them linked up and interwoven with each other thus forming a network. From the very beginning, mitoses were found all over the surface of the tendon explants. There were, however, very little free cells and on replantation their number decreased still further and, sooner or later, they underwent degeneration and disappeared completely.

A six- to seven-day culture usually showed signs of aging; growth and reproduction of cells slowed down considerably and in the migrating cells, degenerative changes became evident.

After eight to ten days of culture — and in some specimens even earlier — the cells lost their functional stability; growth and multiplying had ceased completely. The cells had become rounded and both in the nuclei and the cyto-

plasm degenerative changes had developed; the cytoplasm had become darker and acquired a coarse texture, the nuclei started to shrink.

These, basically, were the regular features in a tissue culture at the different stages of development.

A tendon specimen explanted into tissue culture after three days frozen storage developed a very clear productive zone of cells around its entire circumference.

After nine days frozen storage the same intensive growth of cells resembling fibroblasts, could be observed. If compared with the cells of the previous culture, these were more oblong; the productive zone was broad and there were but few free cells. Mitoses at different stages of the reproductive cycle were mostly found in the periphery of the productive zone.

In tissue cultures of tendon stored in a frozen state for thirty days a well developed productive zone of cells had formed around each explant.

Specimens of tendon explanted after forty-five days of frozen storage (fig. 4) also grew very well, although the period of latency, i.e. the time between explantation and the appearance of the first migrating cells, was markedly protracted (up to three, even four days). The tendon explants had developed a more or less broad halo of regenerated cells and on its periphery, cells at different stages of mitotic division (pro-, meta-, ana- and telephase), could be distinguished.

In a tissue culture of tendon specimens explanted after sixty days of frozen storage, migrating cells resembling fibroblasts could only be found in isolated sections of the culture. In the productive zone, radially orientated cells united with each other by tree-shaped syncytial projections (fig. 5), could be seen. Their nuclei had regular shape, but very they quickly underwent shrinkage. In the cytoplasm, vacuoles had appeared.

Explantation after ninety days of frozen storage showed growth of fibroblasts only in 21 out of 47 specimens. The period of latency for the start of migration had become as long as six days. Most cells of the productive zone were filled with fat droplets and their nuclei were of irregular shape. The mitotic activity had sharply decreased and amitotic division was seen frequently.

Assessment of these findings proves that the biological activity of the cell elements in tendon tissue can be preserved by freezing at  $-25^{\circ}\text{C}$  and even  $-183^{\circ}\text{C}$  and after three months of storage, provided the specimens are treated with 15 % glycerin prior to conservation. The intensity of growth in tissue culture, however, decreases in direct proportion to the duration of storage. In the microscopic picture described in its dynamic succession, we have been able to register almost the same regular features of growth and multiplying in frozen tendon tissue as can be observed in fresh tendon explants. The only difference lies in the period of latency, i.e. the time between explantation and the first appearance of migrating cells, which is longer in tissue culture of frozen than of fresh explants.





Reports in the literature about the preservation of biological activity of lyophilized tissue are very controversial. Dmochowski et Millard (1950) and Passey, Dmochowski et Lasnitski (1950) found cell growth in vitro in lyophilized tissue. Billingham et Medawar (1952) and Kocharian (1960) confirmed these findings. In experiments with explantation of lyophilized skin specimens, Billingham et Medawar detected growth of epithelial cells, pigmented hair and the appearance of surface pigmentation.

Rey (1962), on the other hand, only reported negative results in in-vitro cultivation of lyophilized embryonic chicken heart tissue; not a single explant showed any signs of growth or migration. On the basis of their experimental studies, Smith (1963) and Mikaelyan (1965) came to the same conclusion. Neither did Bilenko (1962), in his very interesting experiments with lyophilized vessel explants, observe any growth.

Assessment of our own findings also permits to express our judgement on the subject under discussion.

Altogether 14 cultures of 87 tendon explants both treated with 15 % glycerin and untreated prior to conservation by freezing, were carried out.

Between freezing and explantation, the specimens were stored for nine days in phials hermetically closed by melting the glass at the tip under maximum vacuum.

In four cultures with a total of 16 specimens, lyophilized tendon tissue was explanted immediately after extraction from the phial. In another ten cultures with a total of 71 specimens, explantation was carried out 1, 2, 3, 4, 5, 6 and 9 hours after rehydration of the specimens submerged in a warm, isotonic solution.

These experiments showed that lyophilized tendon prior or after rehydration manifested no cell growth in any of the cultures, whether treated with 15 % glycerin before freezing and lyophilization or not. During protracted incubation (up to ten days), the explants did not change their appearance or shape.

As can be seen, our findings are in complete contradiction with the opinion of Kocharian, Billingham, Dmochowski, Medawar, Millard, Passey, Lasnitski and others, who observed cell growth in lyophilized tissue, but coincide with the results of Bilenko, Mikaelyan and Smith who stated that tissue lost its capacity for growing in vitro after lyophilization.

The results of our experiments also permit, with a certain degree of probability, to assume that the functional activity of tendon tissue elements is lost or greatly and irreversibly weakened by lyophilization.

It is quite possible that the absence of growth in the lyophilized explants was due to an impediment specific of tendon tissue culture, but it could have also been caused by imperfect explantation technique.



In conclusion it seems unavoidable to point out that tendon explants treated with 15 % glycerin and then frozen at  $-25^{\circ}\text{C}$  or  $-183^{\circ}\text{C}$ , as well as those only cooled at  $+4^{\circ}\text{C}$  in Kovalenko's glucose-citrate-penicillin fluid, after being placed in a nutritive medium, possess the capacity of cell proliferation.

Under these conditions, behaving like fresh explants, tendon tissue not only preserves its original set-up, but also shows subsequent tissue proliferation based on the multiplying of fibroblasts as manifested by the multitude of cells at different stages of the mitotic cycle, many times exceeding the original dimension of the explant.

We were able to show that the growth potential and the capacity for multiplying was the same in frozen or cooled explants as in fresh tendon specimens. The difference only lay in that growth of cell elements started in fresh tendon 24 hours after explantation with the migration of cells and reached its climax with the appearance of fibroblasts, while cells of tendon tissue cooled at  $+4^{\circ}\text{C}$  took 36 hours and those of tissue frozen at  $-25^{\circ}\text{C}$  and  $-183^{\circ}\text{C}$  even from 36 to 48 hours to adapt themselves to tissue culture conditions.

Investigation of the state of tendon tissue conserved by cooling at  $+4^{\circ}\text{C}$  or freezing at  $-25^{\circ}\text{C}$  or  $-183^{\circ}\text{C}$  also disclosed that viability of cells decreased in proportion to the duration of storage. Growth of fibroblasts ceased in cooled tissue after 18 to 21 days and in frozen tissue after three months. In cultures of lyophilized tendon tissue, we were unable to see any cell growth at all. In tendon explants frozen at  $-25^{\circ}\text{C}$  without preceding treatment with 15 % glycerin, only in very rare instances could we observe growth of cells, so that it practically could not be taken into account.

The temperature of conservation, the duration of storage and the addition of glycerin as a substance capable of increasing the general resistance of cells against various injuries are, therefore, the essential factors in the preservation of biological activity of tendon tissue.

#### SUMMARY

The author investigated the viability of cooled, frozen and lyophilized tendon by explantation in tissue culture.

Altogether 78 experiments with a total of 754 specimens of tendon tissue were carried out.

Explants of tendon treated with 15 % glycerin and frozen at  $-25^{\circ}\text{C}$  or  $-183^{\circ}\text{C}$  as well as those cooled at  $+4^{\circ}\text{C}$  in Kovalenko's glucose-citrate-penicillin fluid proved to be capable of cell proliferation, when placed in a nutritive medium. Survival of the cells, however, decreased in proportion with the duration of storage; cell growth ceased completely in cooled specimens after 18 to 21 days and in frozen specimens after three months of storage.

Lyophilized tendon tissue showed no cell growth at all under tissue culture conditions.

In tendon explants frozen at  $-25^{\circ}\text{C}$  without preceding treatment with 15 % glycerin, cell growth was observed but rarely; in two out of 98 specimens.

The author is of the opinion that the temperature of conservation, the duration of storage and the addition of glycerin as a means for increasing the general resistance of cells against any injury, are the essential factors in the preservation of biological activity of tendon tissue.

## R É S U M É E

### **L'épreuve de l'activité biologique du tendon conservé à l'aide de la culture des tissus**

N. P. Demichev

L'auteur a soumis à l'épreuve la vitalité du tendon conservé à l'aide du froid, de la réfrigération et de la lyophilisation. En somme, il a entrepris 78 des expériences avec la totalité de 754 des spécimens du tissu tendineux. Les explants des tendons conservés à l'aide de la glycérine en solution de 15% en suite de congélation à  $-25^{\circ}$  Celsius ou  $-183^{\circ}$  Celsius de même que ceux conservés à l'aide de congélation à  $+4^{\circ}$  Celsius dans la solution de Konvalenko-c'est-à-dire glucose-citrate-pénicilline donnaient des meilleurs résultats, étant capables de la prolifération cellulaire placés dans un médium nutritif. Mais pourtant la survie des cellules s'abaissait en proportion avec la durée de la conservation; la prolifération cellulaire était nulle dans les spécimens congelés à partir du 18<sup>ème</sup> à 21<sup>er</sup> jouet chez les réfrigérés à partir du troisième mois de conservation. Le tissu des tendons conservés à l'aide de la lyophilization n'a montré aucune prolifération cellulaire.

Dans les explants du tissu tendineux conservé à l'aide de congélation sans le traitement avec la solution de glycérine de 15%, la prolifération cellulaire a été observée très rarement; dans la totalité seulement dans deux cas de la somme de 98 des spécimens. L'auteur exprime l'opinion que les facteurs les plus essentiels quand à la conservation de l'activité biologique du tissu tendieux sont présentés par la température de la conservation, la durée de la conservation elle-même, l'addition de la glycérine en tant que moyen d'aggrandir la résistance générale des cellules en face du traumatisme.

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

### **Bewertung der biologischen Aktivität konservierter Sehnen anhand von Gewebekulturen**

N. P. Demitschew

Der Verfasser untersuchte die Lebensfähigkeit von gekühlten, gefrorenen und lyophilisierten Sehnen mittels Explantation in Gewebekulturen. Insgesamt wurden 78 Versuche mit 754 Proben von Sehngewebe ausgeführt. Die Versuche ergaben, dass Sehngewebe, das mit 15% Glyzerin behandelt und bei  $-25^{\circ}\text{C}$  oder bei  $-183^{\circ}\text{C}$  gefroren wurde, ebenso Sehngewebe, das bei  $+4^{\circ}\text{C}$  in der Glukose-Zitrat-Penizillin-Lösung nach Kowalenko im Kühlschrank aufbewahrt wurde, die Fähigkeit zur Zellproliferation behielt, wenn es in ein Nährmedium gebracht wurde. Das Überleben der Zellen nahm jedoch entsprechend der Aufbewahrungsdauer ab; ein Wachstum von Zellen konnte bei Gewebeproben, die 18 und 21 Tage im Kühlschrank aufbewahrt worden waren, sowie bei gefrorenen Gewebeproben nach 3monatiger Aufbewahrung nicht mehr beobachtet werden. Lyophilisiertes Sehngewebe zeigte in der Gewebekultur überhaupt kein Wachstum von Zellen.

N. P. Demichev

ASSESSING THE BIOLOGICAL ACTIVITY OF CONSERVED  
TENDON BY TISSUE CULTURE



Fig. 1. Four-day culture of tendon conserved in Kovalenko's glucose-citrate-penicillin fluid for nine days. Stained with haematoxylin-eosin. General view of proliferative zone of cells resembling fibroblasts. Objective 10X, ocular 10X



Fig. 2. Five-day culture of tendon frozen at  $-25^{\circ}\text{C}$  (Without preceding treatment with frost protecting substance) and stored for 49 days. Stained with haematoxylin-eosin. Single migrating cells resembling fibroblasts can be seen. Objective 9X, ocular 7X

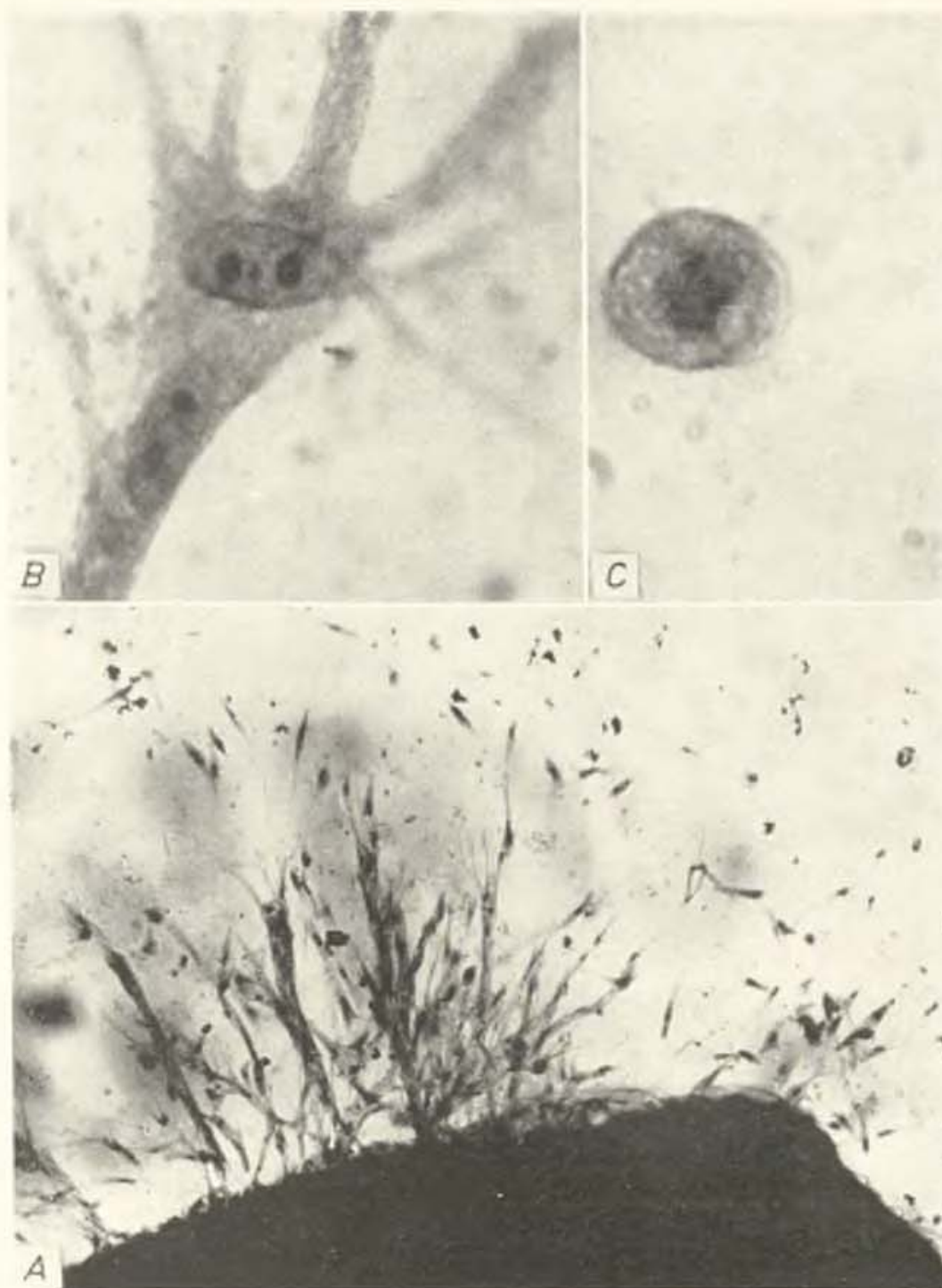


Fig. 3. Three-to-four-day culture of tendon treated with 15% glycerin, frozen at  $-25^{\circ}\text{C}$  and stored for one day. Stained with haematoxylin-eosin — a) general view of proliferative zone. Objective, ocular 10X — b) cell resembling fibroblast of oval shape and with nucleus showing two large nucleoli. Objective 60X, ocular 10X — c) free ("roaming") cell with compressed nucleus. Objective 60X, ocular 10X



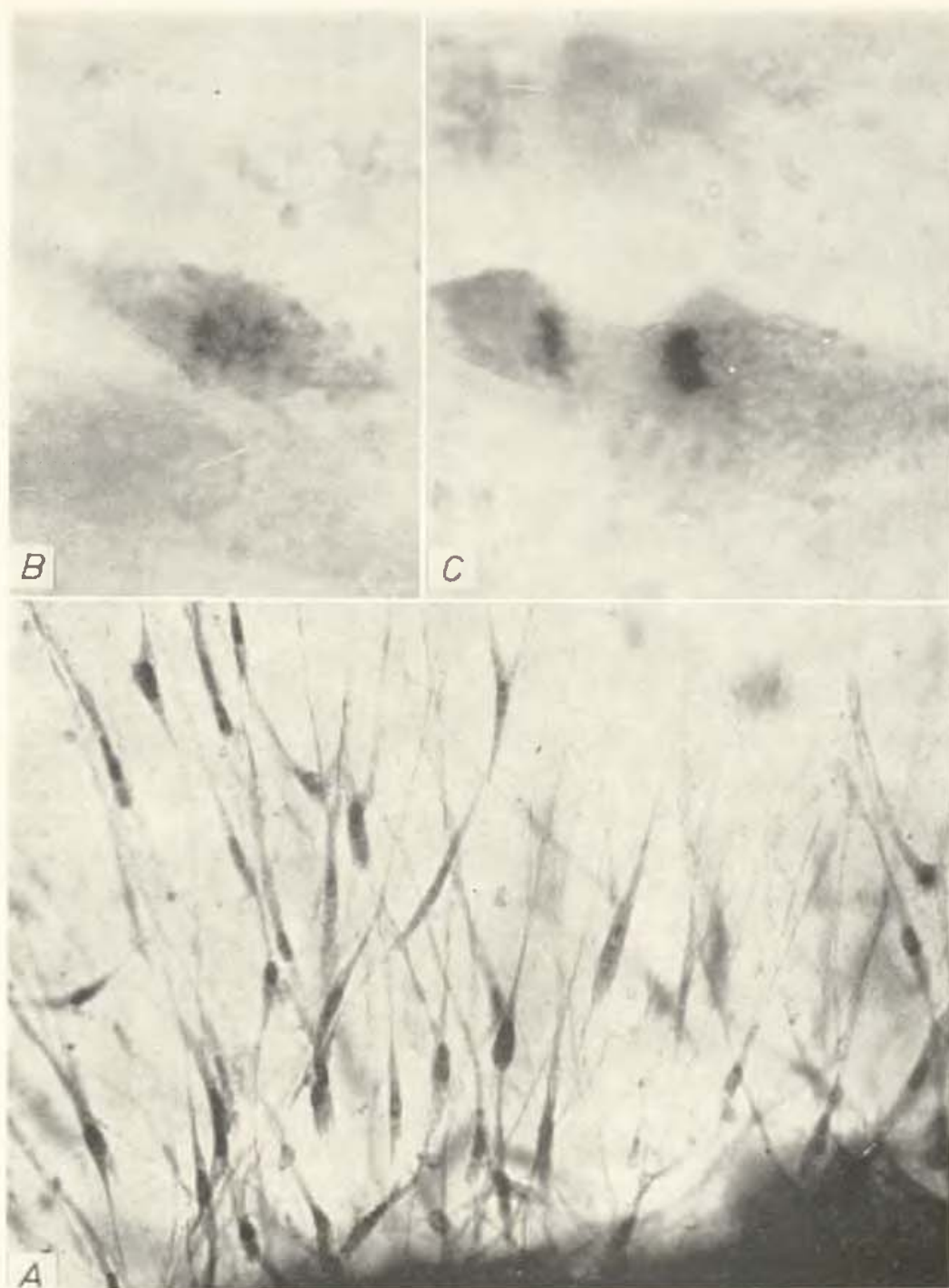


Fig. 4. Five-day culture of tendon treated with 15% glycerin, frozen at  $-183^{\circ}\text{C}$  and stored for nine days. Stained with haematoxylin-eosin: a) general view of proliferative zone. Objective 10, ocular 10X — b) cell resembling fibroblast on mitotic division [metaphase]. Objective 60X, ocular 10X — c) cell resembling fibroblast at the stage of anaphase

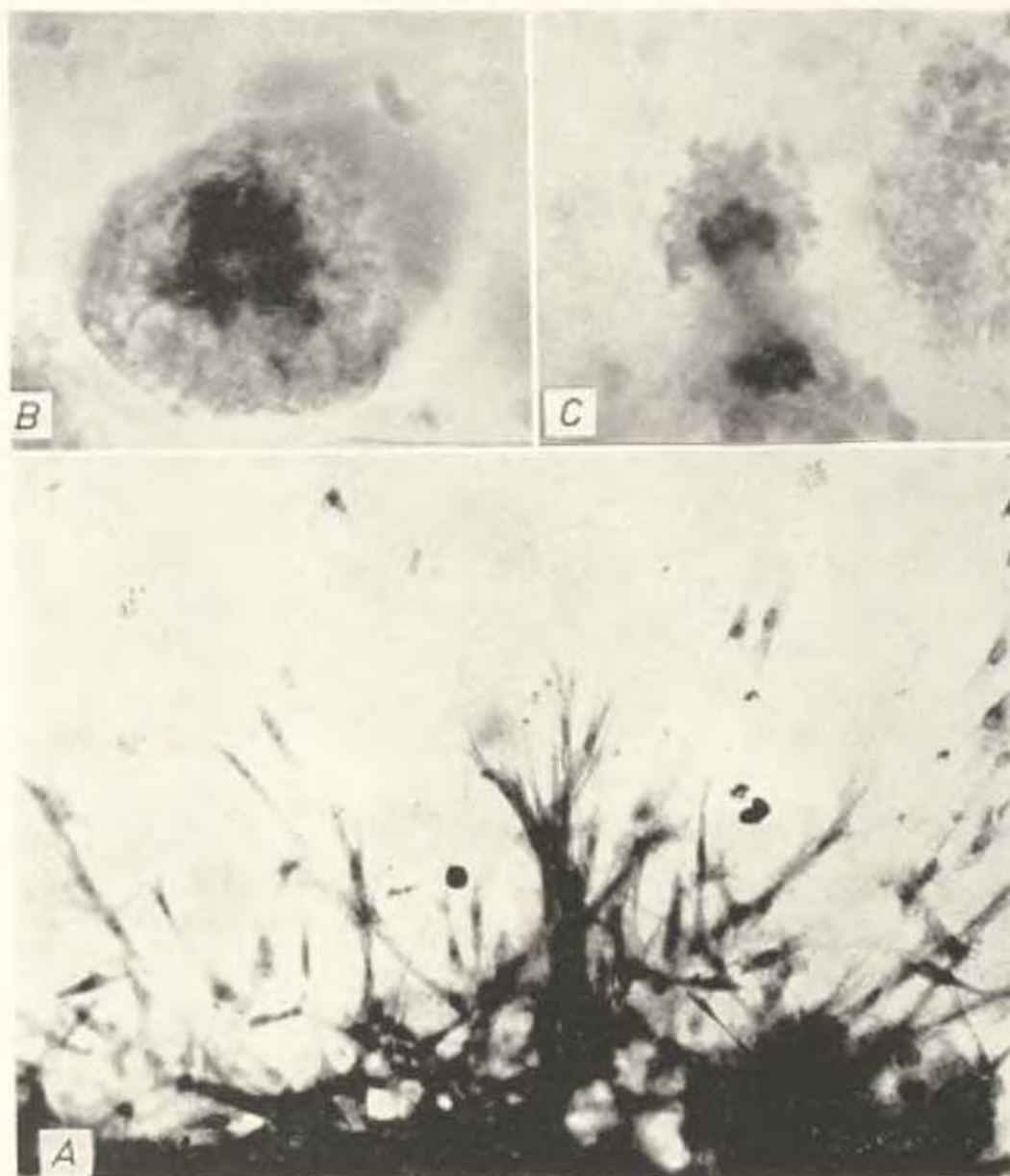


Fig. 5. Four-day culture of tendon treated with 15% glycerin, frozen at  $-25^{\circ}\text{C}$  and stored for 60 days. Stained with haematoxylin-eosin: a) general view of proliferative zone. Objective 10X, ocular 10X — b) and c) cells resembling fibroblasts at different stages of mitotic division (meta- and telephase). Objective 60X, ocular 10X

Sehnengewebe, das bei  $-25^{\circ}\text{C}$  ohne vorherige Behandlung mit 15% Glyzerin gefroren worden war, zeigte nur selten Zellwachstum, nämlich in 2 von 98 Gewebeproben. Der Autor ist der Ansicht, dass die Konservierungstemperatur und die Dauer der Aufbewahrung sowie die Zugabe von Glyzerin, das die allgemeine Widerstandsfähigkeit der Zellen gegen jede Schädigung steigert, die wichtigsten Faktoren für die Aufrechterhaltung der biologischen Aktivität von konserviertem Sehnengewebe darstellt.

## RESUMEN

### La tasación de la actividad biológica del tendón conservado con ayuda de la cultura tisular

N. P. Demichev

El autor investigó la viabilidad del tendón enfriado, congelado y liofilizado por la explantación en la cultura tisular. 78 experimentos con el total de 754 especímenes del tejido del tendón se llevaron a cabo en total. Los explantes del tendón tratados por el 15% de glicerina y congelados a  $-25^{\circ}\text{C}$  o  $-183^{\circ}\text{C}$  tanto como los que fueron enfriados a  $+4^{\circ}\text{C}$  en el fluido de glucoso-citrato-penicilina de Kovalenko fueron capaces de la proliferación celular cuando situados en un medio nutritivo. Pero la supervivencia de las células fue reducida con la duración del almacenaje; el crecimiento de las células cesó completamente en los especímenes enfriados después de 18—21 días y en los especímenes congelados después de tres meses del almacenaje. Los tejidos del tendón liofilizado mostró ningún crecimiento en absoluto bajo las condiciones de la cultura tisular.

En los explantes de tendón congelados a  $-25^{\circ}\text{C}$  sin tratamiento precedente con el 15% de glicerina, el crecimiento de las células fue observado pero con rareza; sólo en dos casos desde 98 especímenes. El autor es de opinión que la temperatura de la conservación, la duración del almacenaje y la adición de la glicerina como el medio de aumentar la resistencia general de las células contra toda la injuria, son los factores más importantes para la preservación de la actividad biológica del tejido de tendón.

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## RESOLUBLE SYNTHETIC MATERIALS IN PLASTIC SURGERY

G. A. ORLOV

During the last decade the use of synthetic materials in the plastic surgery has been widely developed on the purpose to substitute and fix a ligamentary system. The important advantage of these materials lies in the fact that being inserted into the tissues they cause comparatively a slight inflammatory reaction, have no antigenic characteristics and distinguish themselves by a consider strength.

The positive characteristic of slowly resolvable synthetic materials in their ability to form organotropic regenerative tissues as well as substituting tendons and ligaments. Slow and gradual resolving of the material for a few years facilitate a gradual restitution of a function.

In our clinic a netted chemically pure kapron material has been experimentally and clinically tested. This material is being produced for the medical purposes [GOST 3372].

In the experiment the stripes of the synthetic material have been inserted into the layers of the articulation bursa of rabbits. 15 animals have been operated in the sterile conditions. Allotransplantate has been fixed with widely spaced kapron sutures. Suture has been made with a loose tension. The following histological examination of the tissues of the operation region has been made 10—12 days and 5 and 8 months later. The histological sections of the transplantate have been dyed with haematoxylin and eosin and according to the Weigert's method.

In the early period of the observation there has been a moderate productive inflammatory reaction and development of a plane layer of a granulation tissue and a fibroid plate around the transplantate. The fibrous tissue has formed two layers from both sides of the transplantate.

5 and 8 months after inserting of the transplantate there has been found more marked fibrous structure of the plates of the connective tissue. Moreover a newly formed tissue has assumed shape of an additional ligamentary system, fibres of which have passed in parallel to the lines of tension of the articulation bursa.

The thorough examination of an endothelial tissue of synovial membrane under the transplantate showed the tissue to be absolutely intact.

Clinically 27 patients suffering from the habitual luxation of the shoulder-joint have been operated by means of the restoration of the scapulohumeral ligament.

During the operation (endotracheal narcosis with a fluotane or nitrose oxide and myorelaxantes) the anterior surface of the humer articulation (shoulder-joint) has been widely opened. The tendon of the biceps muscle has been strengthened in the intertubercle sulcus.

According to the projection of damaged or atrophied scapulohumeral ligament (from the anterior edge of the shoulder blade to the tubers of the



Fig. 1. Fibrous connective tissue developed around a thread of KAPRON six months after its implantation into joint capsule. Stained with haematoxylin-eosin, magnified with ocular + 10 and objective + 20

humerus) the flap of the capron material of 4,7 cm has been sutured to the articulation bursa with the capron threads. This alloplastic material has been as an imitator of the scapulohumeral ligament and protected the head of the humerus from the anterior dislocation.

The patients have been observed 3 months to 4 years after the operation. In all cases the results of the operation have been found to be satisfactory. There were no recidivations of the luxation. The total volume of movements gradually restored. The patients returned to the manual labour.

Resoluble synthetical material caused no functionaly disorders of the synovial membrane of the joint. It formed sufficiently firm anterior strenghtening of the joint substituting thus scapulohumeral ligament during the movement.

#### S U M M A R Y

Experiments carried out on rabbits showed that connective tissue proliferates around strips of the plastic material KAPRON implanted near a joint which thus reinforces the joint. The synovial lining of the joint remains intact.

On reconstruction of the ligamentous apparatus of the shoulder joint and in order to reinforce its capsule, absorbable KAPRON was sutured into the joint capsule of 27 patients. The connective tissue which then developed around these implants, reinforced the joint capsule sufficiently without impeding joint movements.

#### R É S U M É E

##### **Le matériel absorbable synthétique employé dans la chirurgie plastique**

G. A. Orlov

Les expériences faites sur les lapins ont montré que le tissu conjonctif prolifère autour des mèches du matériel plastique KAPRON implanté dans les alentours des joints causant ainsi l'augmentation de la fixation du joint respectif. La ligne synoviale du joint elle-même reste intacte.

Pour reconstruction de l'appareil ligamentaire de l'articulation scapulo-humérale en but de renforcer sa capsule le KAPRON absorbable fut suturé dans la capsule du joint chez 27 malades. Le tissu conjonctif développé autour de cet implant renforçait la capsule du joint sans que les mouvements soient restreints.

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

##### **Absorbierbare synthetische Stoffe zur Verwendung in der plastischen Chirurgie**

G. A. Orlov

Versuche an Kaninchen ergaben, dass nach Implantation von Streifen aus dem Kunststoff KAPRON in die Nachbarschaft eines Gelenks Bindegewebe um die Kapronstreifen proliferiert und so das Gelenk stützt. Die Synovia-Grenze des Gelenks bleibt intakt.

Bei 27 Patienten wurde bei der Rekonstruktion des Bandapparats des Schultergelenks absorbierbares KAPRON in die Gelenkkapsel vernäht, um so die Kapsel zu verstärken. Das um diese Implantate proliferierende Bindegewebe verstärkte die Gelenkkapsel hinreichend, ohne die Bewegungen des Gelenks einzuschränken.

#### R E S U M E N

##### **Material sintético capaz de absorberse utilizado en la cirugía plástica**

G. A. Orlov

Experimentos con los conejos mostraron que el tejido de comunicación alrededor de las listas del material plástico KAPRON implantado cerca de la articulación prolifera, y así la articulación se corrobora. El contenido sinovial de la articulación se queda intacto.

En la reconstrucción del aparato ligamentoso de la articulación de espalda y para corroborar su cápsula, fue el KAPRON capaz de absorberse suturado en la cápsula de articulación de 27 pacientes. El tejido de comunicación, que se desarrolló después alrededor de estos implantes, corroboró la cápsula de articulación con suficiencia, sin impedir a los movimientos de las articulaciones.

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## GROWTH CHARACTERISTIC OF SKIN THICKNESS IN CHILDREN AND ITS SIGNIFICANCE IN FREE SKIN GRAFTS

N. D. KAZANCEVA

After many years of clinical experience with different modifications of free skin grafts surgeons arrived at the opinion that the best way of closing extensive skin defects is by a dermatome transplant.

One of the main conditions of free skin grafting is an autotransplant with sufficient thickness of dermis. The thickness is the determining factor not only for the immediate but also for the definite result of the operation from the cosmetic as well as functional standpoint. The possibility to use a thick skin transplant without causing greater damage to the donor site depends on the anatomical properties. According to the locality there are either thin or medium grafts containig from one-fourth to three-fourths of the skin thickness [Zoltan 1962].

The thickness of the removed transplant is also decisive for the fate of the donor defect. Its healing depends not only on the regeneration from the edges of the wound but also on the number of the epithelial elements of the skin [sweat and sebaceous glands, hair follicles] which remained in the area after the removal of the transplant. In free skin grafting for extensive defects it is necessary to cut the transplant in such a way as to leave a sufficient quantity of these elements so that the spontaneous healing is assured. Otherwise the newly caused defects on the donor site could endanger the general condition of the patient. To secure fast healing of the donor defect it is necessary to have at last a general idea about the thickness of the skin at the respective place. Already Padgett (1939), the inventor of dermatome transplantations, and later on others as well, discussed the practical importance of this detail.

So far any pertinent discussions of the skin thickness, and its physiological variants, were concerned mostly with the adults [Barker 1951, Southwood 1955, N. E. Postyanoy 1960]; at the utmost, there were only a few reports mentioning that the thickness of the skin depends on growth, sex, nourishment of the individual, and on the functional loading of the skin.

The literary data on the thickness of the skin in children and its changes in growth are very rare. In the Soviet literature there are only the observations



of E. S. Korolev (1902) and M. S. Ass (1953). In foreign literature, according to Möllendorf, there is the more detailed, though by far not exhausting, evidence given by Southwood (1955) and Tsukuda (1957). However these conclusions were not applied in surgical practice.

Obviously the lack of information about the thickness of skin in children is strongly limiting the possibilities of free skin grafting. And up to now, in

Table 1. Characteristic of the observed material.

Age of observed patients	Character of material	
	in post mortem	in biopsy
0— 1 month	9	—
1 month—1 year	30	1
1— 3 years	5	10
3— 7 years	7	7
7—14 years	9	7
25—40 years	6	—
Total	66	25

most clinical papers dealing with dermatome transplantation, the emphasis is put on difficulties connected with the removal of transplants in children which frequently cause prolonged healing of the donor defects.

The necessity of theoretical knowledge of the available and optimal thickness of transplantats in children of different age induced us to a systematic study of the morphology of the children's skin. This observation was carried

Table 2. Thickness of skin in various age groups (expressed in Mi)

Age	Medium skin thickness	Mean square deviation
New borns	764.6	$\pm 49.6$
1— 6 month	760.1	$\pm 24.5$
6—12 month	1132.6	$\pm 16.5$
1— 3 years	1199.5	$\pm 118.2$
3— 7 years	1359.5	$\pm 78.4$
7—14 years	1737.9	$\pm 80.1$
25—40 years	2744.6	$\pm 127.2$

out on those parts of the body which are most frequently used as donor sites (chest, back, outer part of thighs and buttocks.).

In 60 children and 6 adults specimens of skin were taken at post mortem. The same study was concerned with the skin removed during operations in 25 children aged from 10 months to 12 years (six times on the back, four times on the buttock, three times on the thighs, twice on the chest, ten times

on the belly). Altogether 330 specimens of skin were studied. The growth characteristic of the material and the numbers of observations are given in Table 1.

The method of removal and fixation of the material were as follows: a piece of skin, 2 cm<sup>2</sup> in area, was cut off with the subdermal tissue, spread on a stiff paper, and fixated in 20 percent neutral formaline. After fixation the specimens

Table 3. Average number of epithelial elements in skin at different age in lineal square units of a specimen

Age	Average number of adnexa	Mean square deviation
New borns	22	$\pm 1.1$
1— 6 month	20	$\pm 0.8$
6—12 month	17	$\pm 0.9$
1— 3 years	16	$\pm 1.2$
3— 7 years	12	$\pm 0.8$
7—14 years	13	$\pm 0.6$
25—40 years	9	$\pm 0.6$

were mounted in celoidine-paraphine. Hematoxiline-eosine stain according to van Giesson and Masson was used and the elastic fibres were stained by the Weigert's method.

The thickness of the skin and its layers were measured by an ocular-micro-meter, each measuring being carried out at least five times. Thus the mean values of every individual case were attained. At the same time the quantity of epithelial sources in the dermis was ascertained in the area of 5 mm of the preparation. Altogether more than two thousands measurements were carried

Table 4. Healing time of donor wounds in children of various age groups.

Age	Number of patients	Number of donor defects	Mean time of healing in days
0— 1 year	12	22	11
1— 3 years	34	83	14
3— 7 years	47	208	17
7—14 years	38	170	18

out. The results were evaluated by statistical analysis which confirmed the sufficient quantity of the data.

The research of the growth characteristics of the skin architecture revealed that the skin prone to structural changes with age. In the first month of life the epidermis is very thin. The dermal papillae are little differentiated and the skin is, on the whole, thin. Such a skin surface is very easily wounded

Tab. 5. Admissible and optimal thickness of dermatome transplants in children of various age groups (in mm)

Age	Localisation of donor defects				
	Back	Buttocks	Thighs	Chest	Belly
0— 6 months	0.2 —0.3	0.15—0.3	0.15—0.25	0.15—0.2	0.15—0.2
6—12 months	0.25—0.35	0.2 —0.35	0.2 —0.35	0.2 —0.3	0.2—0.3
1— 3 years	0.3 —0.4	0.3 —0.4	0.25—0.35	0.2 —0.3	0.2 —0.3
3— 7 years	0.35—0.5	0.3 —0.5	0.3 —0.4	0.25—0.35	0.25—0.3
7—14 years	0.35—0.6	0.35—0.5	0.35—0.5	0.3 —0.4	0.3 —0.4

and does not seem to protect sufficiently against extraneous disturbing influences. Gradually the number of layers of epithelial cells increases. The horny layer is becoming more massive, the dermal papillae more clearly differentiated, and the collagen and elastic fibres are growing thicker. At the same time the number of cells in the connective tissue is diminishing. All these processes result in a thickening of all skin layers and, consequently, in the increase of the entire thickness of the skin, particularly of the connective tissue layer of the dermis.

The mean data about the skin thickness in different age groups are given in Table 2. It is evident that the skin of newly-born babies is 3.5 times thinner than that of adults. ( $764,6 \pm 49,6$  in children as compared with  $2744,6 \pm 127,2$  microns in adults).

Analysis of the individual findings of skin thickness in newly borns shows that considerable deviations from the average are possible. To a certain degree they depend on the maturity and the nutritive of the infant. After elimination of immature infants of 1.5—2.5 kg of body weight, the thickness of skin in normal newly borns is on the average  $439,1 \pm 37,2$  Mi, in mature infants weighing more than 3 kg  $889,3 \pm 23,6$  Mi, and may attain even 1000 Mi.

The thickness of skin in children from 1 to 6 months old is in fact the same as after the birth. It is on the average  $760,1 \pm 24,5$  Mi, and in some cases even more. In well nourished and mature infants the skin thickness averages 1000—1200 Mi. On the other hand, in infants with poor nourishment, and premature ones, the skin very thin.

In the second half of the first year the skin gets gradually thicker on the average to  $1132,6 \pm 16,5$  Mi. In normally nourished and mature children the skin thickness can attain 1500 Mi in this period. This dependence on nutrition persists over the entire first year of life. While in atrophic infants the thickness of skin is on the average  $542,7 \pm 35,3$  Mi, in normally nourished children it is  $914,8 \pm 65,3$  Mi.

Further growth is accompanied by a further increase of skin thickness; in infants it is usually 1000 Mi (exactly  $1199,5 \pm 118,2$  Mi), in children of pre-school age it approaches 1500 Mi ( $1375,5 \pm 78,4$  Mi), in some cases even 2000 Mi. In children of school age the thickness of skin increases on the average to  $1737,9 \pm 80,1$  Mi, and may attain 2500 Mi respectively. Nonetheless the skin of 14 years old children is thinner than in adults.



We did not follow the connexion between the quality of diet and skin thickness in teenagers, not even in those particular cases which were showing kachexia. We did not observe any changes of skin thickness in insufficiently nourished patients, otherwise in good condition. We did not find any differences between boys and girls under 14 years while in adults there is a difference owing to sex.

Our results are undoubtedly of practical importance for measuring on different parts of the body. In our opinion the greatest thickness is found on the back, the buttocks and on the thighs. These parts must be, therefore, used with preference as donor sites, especially in newly-born infants. Simultaneously with the measuring of skin thickness from various localities we carried out a calculation of the number of skin adnexa in 5 mm of each specimen. The results are shown in Table 3. It is evident that the number of adnexa in the new borns is 2.5—3 times greater than in adults. This higher number seems to be at the expense of hairiness, mainly of hair follicles and their anlage. During growth the number of epithelial adnexa diminishes. The healing time by epithelisation depends on the number of epithelial adnexa in the remaining dermis and, therefore, the potential healing ability of donor defects in children (especially small ones) is greater than in adults. This is confirmed by results in healing 483 donor areas after removal by dermatome in 131 children aged from 1 month to 14 years (Table 4). According to age the thickness of dermatome transplants varied from 0.15 to 0.3 mm. The table shows that the donor wounds in younger children healed much quicker than in older ones.

The comparison of the number of skin adnexa in various localities showed that their distribution on the body surface is as unequal as is the thickness of the skin. The greatest number of adnexa is in places with the thickest skin. The back skin is especially rich in epithelial elements. From the age of three years the number of adnexa in this locality becomes permanent whereas on other places it decreases with growth.

Considering the mean indicators of skin thickness after having measured 325 specimens of skin in 85 children of different age we are under the impression of being able to advise the following thickness of the dermatome transplants for grafts in burnt children (Table 5). The minimal thickness of the transplant in infants is 0.15 mm, the maximal 0.3 mm. In children of school-age it is from 0.3 to 0.6 mm, the same as in adults.

The morphological observations indicate that a free skin graft taken by a dermatome is possible and corresponds with the skin thickness in children of all age groups. The quantity of epithelial adnexa in the skin of small children is greater in comparison with that in adults which safeguards a rapid healing of the donor wounds. Taking in account anatomical peculiarities of the skin structure it is indispensable to take special care when removing transplants in exhausted children in the first year of their life, their skin being especially thin. Without considering age the back is the most suitable donor site in all age groups owing to its thickest skin with the maximal number of adnexa.

## SUMMARY

Microscopic study of 350 skin specimens in 85 children and 6 adults yielded mean informations about the thickness and age characteristics of the skin architecture in areas which are most frequently used by dermatome transplantations for free skin grafts. The admissible and optimal thickness of dermatome transplants in children of various age groups in relation to the locality of the donor site is recommended.

## RÉSUMÉ

### **La caractéristique de croissance de l'épaisseur de la peau enfantine et son importance à l'égard du transplant libre cutané**

N. D. Kazanceva

En suite des examens microscopiques des 355 des morceaux cutanés provenant d'une groupe de 85 des enfants et de 6 adultes les auteurs ont gagné des données moyennes touchant l'épaisseur et les spécificités de l'âge dans la structure de la peau des localités dont on se sert le plus souvent dans la plastie à dermatome faite du transplant libre de la peau.

Une épaisseur convenable et optimale du transplant au dermatome chez les enfants de diverses groupes d'âge en relation avec la localization du «donnor site» vient d'être présentée.

## ZUSAMMENFASSUNG

### **Die Wachstumcharakteristik der Hautdicke bei Kindern und ihre Bedeutung für die freie Hauttransplantation**

N. D. Kazanceva

Auf Grund mikroskopischer Untersuchung von 355 Hautteilchen bei 85 Kindern und 6 Erwachsenen sind Durchschnittsangaben über die Dicke und die altersbedingten Zusammensetzungssonderheiten der Haut an den bei der Dermatoplastik zur freien Hautübertragung häufigst angewendeten Stellen gewonnen worden.

Es wird die zulässige und optimale Dicke der Dermatomentransplantate bei Kindern verschiedener Altersgruppen in Abhängigkeit von der Lokalisierung der Spendegegend empfohlen.

## RESUMEN

### **La característica del crecimiento del espesor de la piel en los niños y su importancia para el trasplante libre de la piel**

N. D. Kazanceva

Sobre la base del examen microscópico de 355 pedazos de la piel en 85 niños y 6 adultos se ganaron las indicaciones medias sobre el espesor y las particularidades de edad de la composición de la piel en los lugares con mucha frecuencia usados en la plástica dermatoma para el trasplante libre de la piel.

Es recomendable el espesor admisible y óptimo de los trasplantes dermatomos en los niños de varios grupos de edad en la dependencia de la localización de la zona donar.

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## THE PROBLEM OF REVASCULARIZATION OF FREE SKIN AUTOGRAFTS

J. ŠMAHEL

Numerous studies dealt with the problem of healing of a free skin autograft, which represents the most frequently used transplantation technique in plastic surgery. Attention was particularly focused to the problem of revascularization. The interest of this aspect of graft healing is comprehensible. In a free skin transplantation the graft is completely deprived of the nutrition supplied by blood vessels and its fate seems to be dependent in the first phase on the formation of vascular anastomoses at the recipient site, as well as on the re-establishment of blood flow within the graft. The authors of numerous studies differed in their opinion. Their views were not uniform and led to the formulation of two distinct conceptions. According to the first conception connections are formed between the proliferating capillaries of the wound bed and the vessels of the graft resulting in the utilization of the original bed of the graft. According to the second conception revascularization is accomplished by the growth of the vessels of the bed into the graft and by their subsequent differentiation into a new vascular system. The question whether an utilization or a destruction of the original vessels within the graft occurs, is closely connected with the broader problem of the survival or replacement of the graft tissues. The acceptance of the first or of the second conception without any reservation would lead to difficulties whenever an attempt would be made at an explanation of accidents encountered in clinical and experimental practice.

In practice the healing of a graft is never uniform and the surgeon has the opportunity to observe a wider range of results varying from a perfect healing during which the graft retains fully the characteristics of a skin over desquamations differing in extent and depth, up to a complete destruction and rejection of the graft. This fact alone shows that revascularization does not proceed always in the same way.

At the Laboratory for Histology of the University Clinic of Plastic Surgery at Prague the problems of the healing of free skin autografts have been studied in recent years. Several hundreds of full thickness skin grafts transplanted to





Fig. 1. A frozen section both of the bed and graft, 4th day after implantation, stained with hematoxylin-eosin, the vessels are injected with a mixture of gelatine and Indian-ink. X50. The lowest part shows the tissue of the bed which is highly vascularized. Through the newly formed anastomoses the injected medium begins to penetrate into the vessels of the graft. — Fig. 2. A histologic section of a healed graft, 8 days after implantation. Stained with hematoxylin-eosin-saffron, X 40. On the lower margin the tissue of the bed.

rats were used for the study of various aspects of the problem. These experiments provided the possibility to investigate the two above mentioned modes of revascularization, i.e. by the utilization of the original vascularization of the graft and by the growth of new vessels into its tissues.

The results obtained confirm that both conceptions are justified and thus contribute to overcome the still prevailing marked discrepancies of opinions concerning this question. This rises simultaneously the question about the mutual relations of both types of revascularization. The comparison of regenerative vascular processes in an open skin defect and in a defect covered by a free skin graft proves as an adequate methodical approach. The comparison showed that at first there is practically no difference. In both instances the defect bed shows a marked proliferation of capillaries leading to the development of a dense vascular network.

On the second or third day vascular buds begin to grow into the cavity of the defect, i.e. towards the implanted graft. The comparison leads to the conclusion that the studies of the revascularization of a free skin graft, actually represent an assessment of processes which are specific for the healing of a not covered skin defect. The graft effects only a modification of these processes and exerts a more or less pronounced favourable effect. This conception of the problem follows from the fact that a transplantation is a procedure performed by man, which has no parallel in the nature and therefore the recipient organism has not at his disposal a firmly established specific response to this situation.

The capillaries growing from the defect bed either form connexions with vessels in the bottom of the graft or they continue to grow into its tissues. It is therefore not possible to consider both types of revascularization, i.e. the utilization of the original vascular system, and the growth of new vessels, as

representing two parallel possibilities occurring promiscuously, or to formulate one of them as a generally valid biological principle. Both distinct types of revascularization represent merely two phases or subsequent stages of a single process and show a mutual dependence. Within three days after the infliction of the defect and implantation of the graft, either the capillaries growing from the bed have formed connections with the functionally well preserved vessels of the graft and in this case further regeneration processes within the bed are inhibited and do not continue to develop and thus the vessels cannot grow into the graft. When connexions between the bed and the graft are not established early enough and in a sufficient extent, or when the vessels of the graft are no more capable of function, then the regenerative vascular processes within the bed are not inhibited and progress further and from the 4th day onward begin to grow into the graft. These interrelations of both types of revascularization, however, do not exclude, but just on the contrary explain the possibility — which is relatively frequent especially in larger grafts — of a combination of both types of revascularization in a single graft, so that revascularization proceeds in some areas by the utilization of the original vascular system, while in other areas new vessels are growing into the graft. The course of healing and the final functional and cosmetic effect of the transplantation depend of course on the way by which the nutrition of the graft was achieved, since the histologic changes occurring within the graft are distinctly related to the mode of revascularization. On the other hand it is possible to draw, with a rather high degree of precision, conclusions about the mode of revascularization, by examining the clinical and morphological pattern.

Under favourable circumstances, in the case of a technically highly skilled transplantation an early establishment of connexions between the proliferating capillaries of the bed and the vessels of the graft occurs (Fig. 1). Revascularization



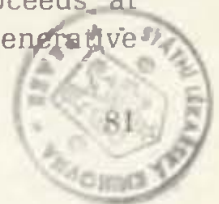
Fig. 3. The boundary between the bed and graft, 20 days after implantation. Stained with hematoxylin-eosin, X 130. The lower part shows the tissue of the bed, the upper part the corium of the graft. They are separated by a band of connective tissue. — Fig. 4. Histologic section of the superficial part of the graft, 16 days after transplantation. Stained with hematoxylin-eosin-saffron, X 100. The lower part shows the well preserved corium of the graft, the upper part the newly formed connective tissue and epidermis.





Fig. 5. A frozen section of the bed and the bottom of the graft, 4 days after implantation. Stained with hematoxylin-eosin, the vessels are injected with a mixture of gelatine and Indian-ink,  $\times 40$ . The bed (the lower half of the figure) contains numerous coiled vessels. Large numbers of buds grow from these vessels into the the fibrin layer (dark band) which separates the bed from the tissue of the graft (at the top of the figure). — Fig. 6. A frozen section of the bed and graft, 9 days after implantation. Stained with hematoxylin-eosin, the vessels are filled with a mixture of gelatine and Indian-ink,  $\times 25$ . A more advanced stage than on Fig. 5, the vessels have already grown into the graft tissue. The dark staining in the area of the growing vessels is due to a marked cellular infiltration.

proceeds at a rapid rate and an adequate nutrition of the graft is restored within 5—7 days after the transplantation. In these instances the margin between the graft and the bed is effaced. The graft shows no substantial histologic changes. There is a regeneration of the epidermis with subsequent desquamation. However a rapid regeneration starting in the margins of the defect and in the appendages of the skin is observed. The functional and cosmetic effect of the transplantation is excellent. (Fig. 2.) Whenever a close contact between the bed and the bottom of the graft is not obtained, e.g. due to a thin layer of fibrin, the capillary buds grow at first through this layer which becomes organized. In this case the original vascular system of the graft is equally utilized for its revascularization, but only after a certain delay. The histologic pattern is characteristic (Fig. 3). Between the bed and the graft lies a layer of newly formed connective tissue with a striking parallel arrangement of collagen fibres. The delayed onset of revascularization of the graft exerts in addition an adverse effect on the survival of its tissues. The epidermis and less or more extensive parts of the papillary layer of the corium undergo necrosis and desquamation; a substantial part of the corium and of the skin appendages, however, remains normal. In a healed graft (Fig. 4) a superficial thin layer of newly formed connective tissue differs from the normal corium by its arrangement and by the increased cellularity. The renewed epidermis is hyperplastic. The clinical effect of transplantation remains satisfactory but the healed graft has not the usual skin relief. When only the growth of vessels from the bed into the graft takes place (Fig. 5, 6), then the result is not very satisfactory. The revascularization effected by the growth of vessels into the graft begins later, proceeds at a slower rate and is always accompanied by more or less extensive degenerative



changes within the graft, leading to the loss and desquamation of both the superficial and deeper layers. The healed graft (Fig. 7) is fibrous, thin and mostly without any skin appendages. Clinically it has a strikingly smooth, glossy and pale surface.

In a complete transplantation failure the vessels of the bed and of the regenerative blastema fail to grow into the graft, but gradually lift it (Fig. 8). After the rejection of the necrotic graft, which in this case fulfilled only the function of a biological dressing, the defect is filled with granulation tissue.

The histologic pattern shows that the revascularization, which was realized by the utilization of the original vascular system of the graft, is most favourable both with regard to its course and to the final result, since the associated histologic changes are no substantial and it is only this type of graft healing which provides a fully adequate substitution for the lost skin. It is justified to designate a revascularization of a graft by utilizing its original vascular system as a healing *per primam*, while the growth of vessels into the graft can be described as a healing *per secundam* or *per defectum*.

In order to answer the question which of the two types of revascularization occurs more frequently it is necessary to consider the conditions under which the first or the second type of revascularization occurs. An indispensable prerequisite for revascularization of the graft by the utilization of its original



Fig. 7. Histologic section of the graft, 12 days after implantation. Stained with hematoxylin-eosin, the vessels are filled with a mixture of gelatine and Indian-ink, X 40. At the lower margin the tissue of the bed. Above it a well preserved layer of corium of the graft without skin appendages which is covered already by the epidermis. At the top a necrotic desquamating portion of the graft. — Fig. 8. A frozen section of the defect filled with granulation tissue, 12 days after infliction of the defect and subsequent implantation. Stained with hematoxylin-eosin, the vessels were filled with a mixture of gelatine and Indian-ink, X 40. Characteristic parallel course of the vessels in the granulation tissue. At the upper margin a residuum of necrotic graft.



vascular system is a skilled technique of transplantation, in particular a close contact of the graft with the bed, as well as a satisfactory biologic condition of the graft. Therefore this type of revascularization makes great demands on the technique and tactics of the transplantation procedure and is also more vulnerable compared to the second mode where the vessels grow through the fibrin layer or some other obstacle, into the tissue of the graft. We believe that the mode by which revascularization and healing of the graft proceed both in experiments and in clinical practice show a dependence on a harmonious interplay of a whole series of factors, among which the technique of transplantation is of primary importance. The experimental and clinical findings indicate that a revascularization of the graft by the utilization of its original vascular system, as well as a preservation of all essential structures of the transplanted skin is possible from the biological view. It will be the task of further research, as well as of experience gained in the daily practice, to achieve that this biological mode of revascularization will be actually commonly realized in all transplantations.

#### S U M M A R Y

The study of revascularization in free skin autotransplants is concerned mainly with evaluation of events specific for healing of skin defects. Capillary buds, growing out of the base of the defect link up, in favourable cases, with the original vessels of the graft. If this link-up, for whatever reason, fails to take place, they continue their growth into the graft tissue. Both distinct types of transplant revascularization represent only two phases or are chronological sequences of stages of a single process. Histological changes occurring in the transplant are obviously dependent on the mode by which revascularization is taking place. Histologic changes in cases of transplant revascularization by utilization of its original vascular bed are unsubstantial, in the presence of mere ingrowth of new vessels into the graft extensive degenerative and necrotic processes take place in its tissues. The mode by which revascularization and healing of grafts takes place in experimental and clinical practice appears to depend on harmonious interplay of a whole series of factors, pride of place being taken by the tactics and technique of transplantation.

#### R É S U M É

##### **Le problème de la révascularization du transplant cutané libre**

J. Š m a h e l

En étudiant la révascularization du transplant cutané libre on examine en somme l'efficacité des données spécifiques pour la guérison du défaut cutané. Les rejetons capillaires partant de la base du défaut se joignent dans des cas favorables aux vases d'origine du transplant respectif. Si cette jonction ne trouve pas lieu faute de diverses causes, ces capillaires continuent à pénétrer dans le tissu du transplant respectif, ces deux manières de la révascularization ne représentent que deux phases ou bien encore deux détails successifs temporaires d'un procès identique. Les changements histologiques qui trouvent lieu dans le transplant dépendent évidemment de la manière de la révascularization du transplant en question. Au cas de la révascularization du transplant

à l'aide de son résaut vasculaire d'origine les changements histologiques ne sont pas essentiels tandis que au cours de la révascularization à perforation de nouvelles capillaires dans le transplant respectif ces tittus sont a soumis à des procès extensifs de dégénération et de nécrose. La manière de la révascularization et la guérison complete du transplant respectif dans l'expériment de même que dans la clinique semble être dépendante d'une coexistence favorable de tout un tas de facteurs dont la plus importante semble être la tactique et la technique de la transplantation elle même.

## ZUSAMMENFASSUNG

### **Das Problem der Revaskularisierung des freien Hautautotransplantats**

J. Šmahel

Beim Studium der Revaskularisierung des freien Hautautotransplantats handelt es sich im Grundsatz um eine Auswertung von Vorgängen, die für den heilenden Hautdefekt spezifisch sind. Kapillartriebe, die aus der Defektbase emporwachsen, schliessen sich im günstigen Falle an die ursprünglichen Gefässe des Pfropfens an. Wenn diese Verbindung aus verschiedenen Gründen nicht zustandekommt, setzen sie ihr Wachstum in das Gewebe des Pfropfens fort. Die beiden unterschiedlichen Wege der Revaskularisierung des Transplantats sind nur zwei Phasen oder zeitnässig nacheinander folgende Stadien eines und desselben Prozesses. Histologische Veränderungen, die in dem Transplantat stattfinden, zeigen eine deutliche Abhängigkeit von dem Weg, auch welchem die Revaskularisierung verläuft. Bei der Revaskularisierung des Transplantats auf dem Wege der Ausnützung des ursprünglichen Gefässflussbettes sind die histologischen Veränderungen unwesentlich, beim einfachen Durchwachsen neuer Gefässe in den Pfropfen verlaufen in seinem Gewebe umfangreiche degenerative und nekrotische Prozesse. Der Weg, auf welchem die Revaskularisierung und Zuheilung des Pfropfens in der experimentellen und klinischen Praxis verläuft, scheint von einem günstigen Einklang einer Reihe von Faktoren abhängig zu sein, unter welchen die taktische und technische Durchführung der Transplantation an erster Stelle zu nennen ist.

## RESUMEN

### **El problema de la revascularización del autotransplante libre de la piel**

J. Šmahel

En el estudio de la revascularización del autotransplante libre de la piel se trata en substancia de la valorización de las acciones específicas para el defecto de la piel cicatrizandose. Los retoños capilares crecientes de la base del defecto, se reunen en caso favorable a los vasos originales de la grieta. Si esta unión no se realiza de varios razones, continuan en su crecimiento en el tejido de la grieta. Los dos casos diferentes de la revascularización del transplante son solamente dos fases o estadios cronológicos del mismo proceso. Los cambios histológicos que se realizan en el transplante, manifiestan una dependencia distinta en el modo del cual se realiza la revascularización. En la revascularización del transplante por aprovechar su cauce vasculoso original, los cambios histológicos no son considerables, cuando los vasos nuevos solamente entretejen en la grieta, se tratan en sus tejidos extensos procesos de degeneración y necrosis. El modo, con el cual se realiza la revascularización y la cicatrización de la grieta en la práctica experimental y clínica, parese dependiente de la concordancia favorable de toda la línea de los factores, de los que en el primer lugar es necesario indicar la realización de la transplatación táctica técnica.

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TABLE OF CONTENTS

Quigley, Jr. L. F., Cobb C. M.: Clinical and Subjective Evaluation of Speech	1
Šurina I., Jágr J.: Action Potentials of Levator and Tensor Muscles in Patients with Cleft Palate . . . . .	21
Marino A.: Reconstructive Plastic Surgery in Extensive Perforations of the Nasal Septum . . . . .	31
Alexandrov N. M.: Resection of the Upper Jaw with Elements of a Primary Plastic . . . . .	38
Klen R., Heger J.: A Contribution to the Degradation of Skin and Cornea Preserved Hemibiotically in the Temperature Near 0 °C (Brief Communication) . . . . .	47
Demichev N. P.: Assessing the Biological Activity of Conserved Tendon by Tissue Culture . . . . .	56
Orlov G. A.: Absorbable Synthetic Material Used in Plastic Surgery . .	67
Kazanceva N. D.: Growth Characteristic of Skin Thickness in Children and its Significance in Free Skin Grafts . . . . .	71
Šmahel J.: The Problem of Revascularization of Free Skin Autografts .	78

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