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SANDRA LYNN ETTEMA

ENTITLED A QUANTITATIVE HISTOLOGIC STUDY OF THE

NORMAL HUMAN ADULT SOFT PALATE

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF BACHELOR OF SCIENCE

Instructor in Charge

APPROVED:

HEAD OF DEPARTMENT OF SPEECH AND HEARING SCIENCE

A QUANTITATIVE HISTOLOGIC STUDY OF THE NORMAL HUMAN ADULT SOFT PALATE

By

Sandra Lynn Ettema

Thesis

for the
Degree of Bachelor of Science
in
Liberal Arts and Sciences

College of Liberal Arts and Sciences
University of Illinois
Urbana, Illinois

1991

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my research advisor, Dr. David P. Kuehn, for his suggestions, guidance, and encouragement. I have learned a great deal from this experience and with his knowledge and organizational input, I have achieved a long desired goal of mine. During the course of my research study, I had encountered countless questions and problems. Dr. Kuehn and I grew very close as he lead me through each of my obstacles. I feel he is not just my advisor, but also my personal friend.

I would also like to thank Dr. Bilger for the use of his resources and his personal input. It is great to have professors who care and take the time for their students.

I would also like to extend my sincere appreciation to Brian Wills, Kevin Hammann, Justin Thompson, and Sarah Fedder, who took time out of their busy schedules to aid in my research project. I greatly appreciate their time, input, and support.

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CHAPTER I

INTRODUCTION

To learn about pathologic mechanisms of the upper pharynx, one must first have a complete basic understanding of the intricacies involved in the normal mechanism. Professionals in this field require precise calculations of tissue components of the soft palate to construct a functional biomechanical model of the palate to use in instruction and/or for risk-free surgical simulations. Anatomic investigations of the velopharyngeal region reflect general information obtained from gross dissections of this area. The best way to study detailed anatomy of the velum is by using histologic techniques (Kuehn and Kahane, 1990).

Histologic studies of the velopharyngeal region using light microscopy have only provided general information about the tissue components of the soft palate and surrounding structures. For example, it is

known that the anterosuperior region of the velum consists of tendinous tissue, the anteroinferior region consists of glandular tissue, and the central portion consists of muscular tissue (Kuehn and Kahane, 1990). Much more information is needed concerning the normal anatomy of the velum as a basis for understanding the abnormal anatomy. One way to enhance our current knowledge would be to sort the distribution of muscle fibers in the soft palate and their vectors of pull (Dickson, 1975). If more detailed information of the soft palate could be obtained (i.e. quantification of specific tissue types and calculating fiber density and angulation), then accurate identifications of standard patterns in tissue compositions could provide the needed information to construct a precise surgical model to compare and contrast the cleft and normal mechanisms.

There is an increasing number of health professionals directly involved in the care management of the cleft palate. This histologic research will have practical application in the medical fields where clefts are dealt with in a routine fashion such as Plastic Surgery, Craniofacial Surgery, and

Otolaryngology (Jesiolowski, 1987). Cleft palate surgery requires reconstruction of a pathologic soft palate to resemble and function as close as possible to a normal velum. It is known that the levator veli palatini muscle is essential for normal velopharyngeal closure (Boorman and Sommerlad, 1985). Correction of the abnormal levator in cleft palate patients requires an indication of how far the levator muscles must be moved to come as close to normal functioning and structure as possible. It is extremely important to understand the anatomy in both the normal and cleft palate subject to achieve optimal muscle correction (Boorman and Sommerlad, 1985). To follow through with the above, a thorough understanding of tissue distribution and quantification plays an important role in reconstructive procedures.

There have been extremely limited reports using a systematic histologic approach. Reports concerning the tissue composition of the normal human soft palate have only provided general information. The primary purpose of this study is to examine and quantify the specific tissue types of ten specimens of normal human adultsoft palates using a systematic histologic approach. A

Personal Computer (PC) - based true-color image analysis system will be used which contains a Grains Mode to calculate the area of tissue components. The collection of more detailed information concerning the velopharyngeal region will enhance our current knowledge as to the quantification and distribution of tissue types in the nonpathologic population. Identification of normal patterns of tissue distribution and amount will establish a foundation for further research concerning the structure and function of the soft palate.

CHAPTER II

REVIEW OF LITERATURE

It has been noted that most anatomic investigations of the velopharyngeal region have only involved gross dissection. This has lead to a generalized understanding of tissue components in the soft palate. This information is important, but to further advance construction of biomechanical models, more precise data on the velum needs to be gathered. A study conducted by Kuehn and Kahane (1990) was the first step toward detailing the tissue components of the normal human adult soft palate using a systematic histologic approach. This study is reviewed in detail below.

In the Kuehn and Kahane study soft palates were removed in their entirety from ten adult human cadavers, four males and six females. The extracted palates were bisected longitudinally and measured in

length from the most anterior to the most posterior point. The bisected palates then were divided, according to each of their lengths, into ten equal blocks form anterior to posterior. This was done to normalize soft palate length across all subjects. There were no qualitative differences in gross anatomy between right and left halves or between male and female subjects. However, as expected, the male soft palates, at least in one dimension, were somewhat larger than the females, with thickness of the uvula being significantly larger than that of the females.

Only the face ("top" portion) of each of the ten blocks was sectioned and stained, with the use of three different stains. This resulted in a sample of three hundred histologic slides that were examined, and major anatomic characteristics were noted.

The tissue composition of an average adult human soft palate was observed to consist of: 1) the oral aspect, which is mostly glandular tissue with adipose located laterally; 2) the two middle layers consisting of muscular tissue of the transverse levator veli palatini fibers possibly with palatopharyngeal fibers interspersed and the longitudinal musculus uvulae

fibers; 3) a superoanterior layer consisting of the tensor veli palatini tendon; and 4) a posteroinferior layer consisting mostly of glandular tissue. The results of the Kuehn and Kahane study deal primarily with the structure of the velum between the lateral pharyngeal walls and show trends of structural consistency across subjects.

The results of Kuehn and Kahane point in the direction of understanding the structure of the soft palate, but there are many aspects of the anatomy of the velum that deal with its mechanization. First, as noted by Kuehn and Kahane, the soft palate is not a self-contained structure. The lateral boundaries of the velum are difficult to define, and it is obvious that structures lateral to these boundaries are important in the functioning of the velum. However, these were not dealt with in the Kuehn and Kahane study and must be considered to produce an accurate biomechanical model of velar movements and structures. Secondly, although the Kuehn and Kahane study does show some structural consistency across subjects, it has not been determined whether a "generic" velum will function adequately with respect to the central tendency of

variability across subjects. Such within and between subject variability may complicate the construction of a biomechanical model.

To provide the greatest use in construction of biomechanical models, quantified information concerning spatial distribution of tissues and characteristics of fiber density and angulation must be obtained. Recent advances in image analysis systems have put quantification measurements at the forefront and have made them more accessible (Kuehn and Kahane, 1990). An individual study, done by Kurt A. Jesiolowski, under the direction of David P. Kuehn, Ph.D., was begun in 1987 to apply quantification analysis to the specific tissue types located in the soft palate. Jesiolowski calculated quantifications of different tissue types from the one hundred slides stained with the trichrome stain in the Kuehn and Kahane study. These slides were chosen because they proved to be the most sensitive in differentiating most of the major tissue types of interest (Kuehn and Kahane, 1990). To reiterate, the ten slides of every palate were separated by distances of proportional length in relation to each palatal section. Each of these slides was viewed, and the

areas of M-Muscular, G-Glandular, C-Connective, and A-Adipose were recorded. These areas then were converted to percent total tissue to quantify the distribution of all four types across all specimens (Jesiolowski, 1987).

Jesiolowski made use of the Bioquant System IV which is a computerized monochrome image analysis system manufactured by R & R Biometrics, Inc. The Bioquant system is housed in the Department of Cell and Structural Biology, University of Illinois, in the Laboratory of Dr. Patricia J. O'Morchoe. Each slide was placed on the stage of a Leitz Laborlux D light microscope and viewed under a magnification of 25x. The scope was coupled to a Taxan terminal where it produced a monochrome image (Jesiolowski, 1987). By moving a mouse-controlled cursor, an arrow concurrently followed the same path on the terminal screen. Bioquant left a trace line on the screen around the selected tissue patches of interest. Upon returning to the point of origin, the selected area was expressed in square millimeters. Bioquant was also capable of calculating percentage of total area of desired tissue types. Jesiolowski recorded his data in two ways. A

notebook record was kept as well as the results being stored on the software cartridge of the Bioquant System IV. This was done in attempt to establish patterns of tissue distribution in conjunction with the quantity of tissue (Jesiolowski, 1987).

For quantified measurements to be obtained, it is obviously necessary to define the boundaries of the velum. Two of the boundaries are naturally defined, the nasal surface and the oral surface. A third boundary was clearly specified in relation to the methodology used, that is, the medial edge that resulted from the longitudinal bisection of the velum. The fourth boundary, specifically the lateral margin, is problematic. There is no true anatomic boundary distinguishing the soft palate from the lateral pharyngeal wall because the velar contents blend with the pharynx laterally. Therefore, an operationally defined lateral boundary was calculated for each individual velum (Jesiolowski, 1987). Each of the ten specimens were standardized by the use of the length of the velum (Lv) and width of the uvular base (Wu) expressed as a ratio. The Lv/Wu ratio was calculated for each of the subjects, and the resultant values

then were averaged to obtain a Lv/Wu average value, which was equal to 3.72. The equation was adjusted to 1/2Lv / 1/2Wu average because the prepared cross sections were previously bisected sagittally. This still was equal to 3.72. The adjustment was done to allow for proportional standardization numbers which would be in closer proximity to the working dimensions (Jesiolowski, 1987). The standardization value (J-Unit) was figured for each subject by recalculating 1/2Lv / 1/2Wu using half the original individual velum length values and 3.72 (1/2 Lv original/J = 3.72). Each J-Unit value, therefore, is a variable distance in millimeters that equals half the average width of the uvular base of a soft palate with a certain length (Lv) (Jesiolowski, 1987). These calculations served as the means for quantitatively establishing the lateral limits of each subject by applying two J-Units to every section of each of the ten bisected soft palates. Because the velum had been bisected, the two J-Units represent only one-half the velum, and four J-Units would represent more accurately the total left-to-right dimension of the velum. Two J-Units on either side of the midsagittal plane correspond roughly to the

region where the free margin of the velum blends into the lateral pharyngeal wall. The single J-Unit values for each subject are listed in Table 1 which is taken from Jesiolowski (1987).

The subjects and sections were analyzed according to average tissue percentage. This indicated which tissues were most abundant, where, and to what extent (Jesiolowski, 1987). The individual study begun by Jesiolowski was never completed but provided useful principles of investigation for the current study. The general purpose of the current investigation is to examine and quantify the specific tissue types of the same ten subjects reported in Kuehn and Kahane (1990) and used in Jesiolowski's study, but with the use of a PC-based true-color image analysis system. The area of tissue components T-tendinous, G-glandular, C-connective, M-muscular, A-adipose, and O-other are reported and will aid in hypothesizing relationships between the structure and mechanical function of the velum and velopharyngeal mechanism. This will also provide a basis for construction of functionally useful biomechanical models.

TABLE 1. Single J-Unit values for each subject.

SUBJECTS	1/2Lv original/3.72	J-Unit standardized single value*
M1	18.5/3.72	5.0 mm
M2	20.3/3.72	5.5 mm
M3	20.5/3.72	5.5 mm
M4	21.8/3.72	6.0 mm
F1	18.3/3.72	5.0 mm
F2	15.5/3.72	4.0 mm
F3	23.1/3.72	6.0 mm
F4	19.4/3.72	5.0 mm
F5	17.2/3.72	4.5 mm
F6	17.1/3.72	4.5 mm

*The J-unit values were doubled, and that value was used as the predetermined standardized lateral boundary. The lateral boundary thus determined corresponds roughly to the region where the free margin of the velum blends with the lateral pharyngeal wall.

CHAPTER III

METHODS

Subjects

The specimens used in this investigation were taken from ten normal human adult cadavers, four males and six females. The soft palate was removed in its entirety from each cadaver. One-half of each palate was divided into ten blocks of equal anterior-posterior thickness. The face of each block was stained with a trichrome stain, thus providing a sample of one hundred slides (Kuehn and Kahane, 1990). The same slides used in the Kuehn and Kahane study and the Jesiolowski study also were used in the present investigation.

Equipment

The equipment used to collect and analyze data include the following: a PC-Based (IBM AT) true-color image analysis system (American Innovision, Videometric

150), Electrohome 38-DO51MA-YU graphics screen, single chip-Cohu RGB CCD color camera, light box, and Balplan microscope. Videometric 150 (V150) consists of modes that allow one to organize and collect different types of data, and the utilities are the commands that affect these modes (American Innovision, Inc., 1988). System modes cause the V150 to prepare the data collection environment for acquisition of various types of data. To use data collection modes, the image must first be "grabbed" or stored in computed memory. For the present study, only the modes of Points, Lines, Areas, and Grains were used. Utility commands cause the V150 system to perform an action, such as clearing the screen, calibrating, and/or setting threshold levels. System modes are displayed across the top of the PC command screen and are seen in capital letters when they are activated. System utilities are located in a column along the right side of the screen. Table 2 depicts the command screen display set up. A mouse is used to move the cursor around the screen to activate and/or deactivate a mode or select a utility on the command screen, to switch between the command screen

TABLE 2. PC command screen display format.

POINTS LINES AREAS %trans GRAINS centroid axis shape*	
__Variable__Value__Number__Mean__StdDev	
POINTS***	Set**
LINES	Calibrate
AREAS	Threshold
GRAINS	Frame Buffer
TRANS	Files
	Extensions
	Record
	Clear Screen
	Erase
	Reset Frame
	Reset
	Quit
	Saving: OFF
	Data: AUTO
	Screen Width
	512.00

* Data collection modes
** Utility commands
***Data area - actual data collected in center of screen

and the Electrohome (graphics screen), and to close groups on the Electrohome screen.

The data collection modes used in this investigation include the following: Points, Lines, Areas, and Grains. The Points mode allows the user to count objects in the video image, thus calculating the number of points collected. The Lines mode measures the length or perimeter of an image or set of images. To calculate area of the figures outlined, one activates the Areas mode. The Grains mode is the mode for which the present study has the most interest and is the primary manner for data collection of the different areas of tissue compositions. This mode is not available for a monochrome image analysis system and represents a significant technological advancement over the previous system used in the Jesiolowski (1987) study. The purpose of the Grains mode is to count the total number of pixels within a designated area whose brightness is within a set range of minimum and maximum brightness (i.e. is a specific color). This mode then excludes features in the field that should not be calculated, such as artifacts, border, etc. (e.g.

calculates only area of muscular tissue in the field) (American Innovision, Inc., 1988).

Utility commands used frequently in this study include the following: Calibrate, Threshold, Frame Buffer, Files, Record, Reset Frame, and Data. The purpose of the Calibrate command is to set the number of units contained in each pixel on the screen so measurements will be recorded in meaningful units. After calibration, the screen width value is displayed on the command screen in calibrated units. The Threshold command allows interactive setting of thresholds in luma (i.e. brightness) and specific colors (i.e. hue and saturation). Under the Threshold command one can clear, add, and/or subtract the threshold of color (American Innovision, Inc., 1988). The Frame Buffer command contains a submenu containing utility commands for manipulating the frame buffer (i.e. the video image on the Electrohome screen) such as View Live Scene, Grab Frame, Save to File, Load Buffer, and Create File. The utility command used to name a data file or branch out to other programs from the V150 system is the Files command. There is a submenu of choices for the Files command such as Branch

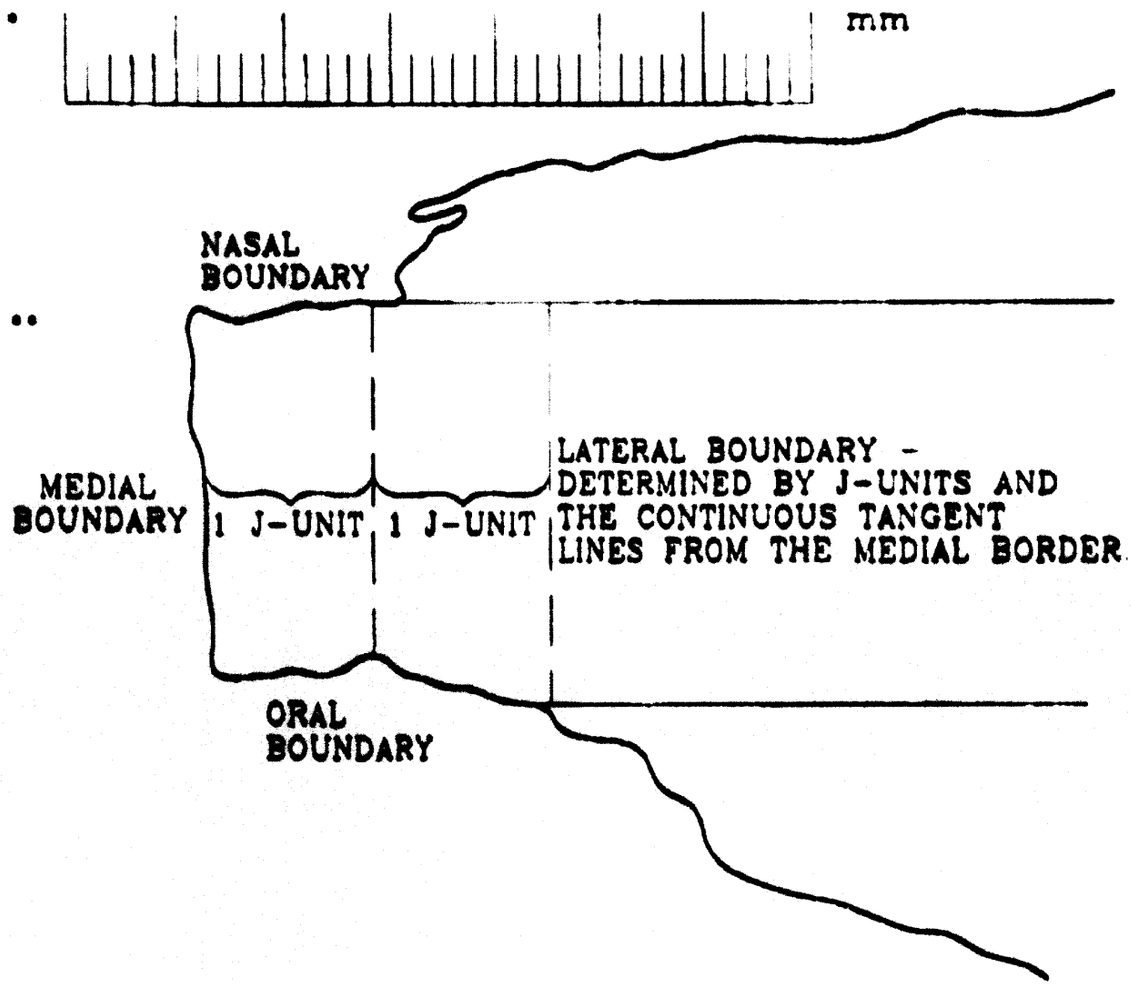
to: command, which allows one to branch to DOS; Saving; and Datafile: filename, which allows one to name the data file. Record allows the user to close a group on a set of data. A group can be closed only if the Data: manual mode is "on." This, in turn, records the data for that group. Reset Frame erases all data which have been collected, zeros variables, and clears the graphics screen. This does not, however, change the current modes in use.

To display a video image on the Electrohome screen, a video camera and light source are necessary. A single chip Cohu RGB CCD color camera was used to view the specimen on the slide. The advantage of using a CCD camera is that it is less subject to geometric distortion than, for example, a tube camera, because the sensors are composed of discrete arrays mounted on a plane surface whereas tube sensors often have uneven or curved sensing surfaces (Spomer and Smith, 1988). The source used to transmit light through the slides was a light box containing fluorescent bulbs covered by a plexiglas diffusion plate. A Balplan microscope, manufactured by Bausch & Lomb, was used to verify tissue composition under a magnification of 4x.

Data Collection

The data were collected in a systematic fashion. Each subject's data were collected individually for each section. One data file was created for each subject and subsequent buffer files were established for each section. The first slide (i.e. section) for a particular subject was imaged and saved in a buffer file. Each slide's buffer file also contained an image of a transparent ruler in mm. The image (i.e. section) of interest that appeared on the Electronic screen then was calibrated, by use of the imaged ruler, to its magnification as viewed through the CCD color camera (Figure 1). Next, the values of area of tissue for T-tendinous, G-Glandular, C-Connective, M-Muscular, A-Adipose, and O-Other tissue components were calculated in that order for each section of a subject. The Threshold was set and reset for each tissue type (i.e. specific color). Then the area containing that particular tissue and encompassing two J-Units was traced and calculated. The ruler, again, was used to measure the distance laterally (i.e. two J-Units) of the soft palate. For example, if the two J-Unit value was 10 mm, then the experimenter would measure the area

Method used to determine calibration measures, J-Units and lateral boundaries.



* Imaged ruler used for calibration and determining J-Unit measures.

** Depiction of a section (i.e. slide) of the soft palate with consideration of the lateral boundary measurement.

of tissue encompassed by the medial, nasal, oral and 10 mm lateral (relative to the medial) boundaries. As the experimenter moved into the latter sections of a subject, the specimen naturally separated where the palatopharyngeus muscle fibers and the salpingopharyngeal fold along the nasal surface and the palatoglossus muscle fibers along the oral surface came into view. The experimenter did not include these upper and lower tissue portions when assessing the lateral boundary (i.e. J-Units) of a section. A tangent line, which corresponded to the length of the two J-Unit value for a particular subject, was drawn in continuation with the most medial aspects of both the nasal and oral surfaces (Figure 1).

Points, Lines, and Areas also were calculated at the same time as particular tissue area (Grains) for each section of a subject. The experimenter then switched to the manual Data mode with the Saving "on" and proceeded to record the data using the Record utility command. These steps were repeated through the rest of the sections and for all the subjects. A new data file was created for each new subject as well as a new buffer file for each new section of a subject.

The experimenter, when reaching the final sections (in or near the uvula), would use the microscope to ensure correct identification of tissue types. This was done because the specimens became quite small in the uvular blocks. Data were recorded in notebook and in the software package of the V150 program. The notebook records also included an estimate of specimen area which used the doubled J-Unit value and the dimension of the specimen from the nasal to oral surface as the area variables. These estimates were compared to the actual area calculations of each section to ensure that the calculated area was within a reasonable range.

Once all one hundred slides were examined and tissue components quantified, the data files for each subject, which contained all ten section data, were copied to a floppy diskette. These data files, in turn, were copied to a Word Perfect 5.0 program in the c: drive. The data files then were manipulated for each section within a subject and for all subjects. The particular data sets (i.e. Points, Lines, Areas, Grains) were recorded in tables labeled T, G, C, M, A, and O tissue components for each section of each

subject. The subject and section number were labeled at the top for easy identification. A sample data collection set is shown in Table 3. These are the actual data collected in the present investigation for Section 1/Female 6. Points for tendinous and glandular tissue resulted in 3 and 10 respectively. Only three points were required to encompass the area of all tendinous tissue, and 10 points were required to encompass the area of all glandular tissue. The parameter "Lines" is the resulting perimeter value of the particular area of interest the experimenter traced. Areas is the resulting field in mm^2 that the experimenter obtained when tracing the field containing a specific tissue of interest. Grains is the actual area in mm^2 for the specific tissue of interest. Grains values are smaller than Areas values because the Grains measures are actual measurements of the highlighted tissue (i.e. specific color) area within the Areas field. In other words, the Areas value is the measurement of the total field traced, and the Grains value is the measurement of total area of specific tissue highlighted within the total field traced.

TABLE 3. Example of data collection set.

SECTION 1/FEMALE 6

	Points	Lines	Areas	Grains
Tendinous	3	25.460	16.576	7.611
Glandular	10	32.177	67.957	20.641
Connective	13	33.299	53.972	16.102
Muscular	16	39.039	72.760	3.971
Adipose	4	41.956	65.048	6.008
Other	18	37.585	72.106	3.516

Data Analysis

Subject F2 was not used in the analysis of the data because this subject possessed an unusually short and thick velum. The ten blocks of tissue, from anterior to posterior, were not accurately sectioned because of the odd structure of this velum. Therefore, the data for subject F2 were not used at all, which left a total of nine subjects for the data analysis. Furthermore, averages were taken only on eight subjects for section 10 due to the extremely small girth of the uvula in subject F6.

Three categories of results were compiled: average area in mm^2 using absolute values, average area in mm^2 using relative values normalized by J-Unit, and average area in percentages normalized across subjects for each section and tissue type.

Average areas in mm^2, using absolute values, were calculated by adding all the results for T, G, C, M, A, and O tissue components for each section and across all nine subjects. For example, T(M1) + T(M2) + T(F1)+...+T(F6) = total tendinous tissue for section 1 across all subjects. This total amount of tendinous tissue then was divided by nine to get the average area of tendinous tissue for all subjects in Section 1. The

same procedure was done for each tissue type in each section and across all subjects.

Average area in mm^2, using relative values normalized by J-Unit, was calculated by figuring the mean J-Unit value for all subjects and then dividing their particular J-Unit values into the mean J-Unit value calculated. This resulted in a ratio of the mean J-Unit value to the subject's particular J-Unit value. Table 4 lists the ratio values calculated for each subject. Multiplication of each subjects's complete data set by this ratio normalized all the results of all the subjects by J-Unit (i.e. lateral boundary). The experimenter then proceeded to calculate as above for absolute values, except using the newly calculated normalized values as the data pool. The purpose of this procedure was to adjust for the operationally defined lateral boundary variance across subjects.

Average area in percentages across subjects for each section and tissue type was obtained by first calculating the total tissue amount for each section of each subject. The total tissue amount for each section then was divided into the specific types of tissue amounts to get a percentage of tissue for that section

TABLE 4. Ratio values used to calculate relative average area in mm^2.

Subject	Mean J-Unit*/Subject J-Unit	Ratio Value
M1	5.22/5.0	1.044
M2	5.22/5.5	.949
M3	5.22/5.5	.949
M4	5.22/6.0	.87
F1	5.22/5.0	1.044
F3	5.22/6.0	.87
F4	5.22/5.0	1.044
F5	5.22/4.5	1.16
F6	5.22/4.5	1.16

*The mean J-Unit value for all subjects was calculated to be 5.22 mm.

and type of tissue. The experimenter performed such calculations for all sections in all subjects, resulting in percentages for each type of tissue in each section for all subjects. Calculations for average percentages across subjects for each section and tissue type were then figured in the same manner as the absolute value calculations above.

CHAPTER IV

RESULTS

The results show definite patterns of tissue distribution and quantity. It is clear that the tendinous tissue is extremely prominent (about 10% of total tissue) anteriorly, and gradually diminishes by section five in all subjects (Figure 2). Qualitative measures from Kuehn and Kahane (1990) show the tensor veli palatini tendon no longer present in section four, but with the present image analysis system, a minute amount of tendinous tissue was still detected in section five.

Of the three major tissue types (i.e. glandular, connective, and muscular), both the glandular and connective tissues appear to be quantitatively uniform (about 22% and 36% respectively of total tissue) in their concentration as one moves posteriorly (Figures 3 and 4). This finding is consistent with that of Kuehn

Figure 2.

TENDINOUS TISSUE
Area Ave./Percentages

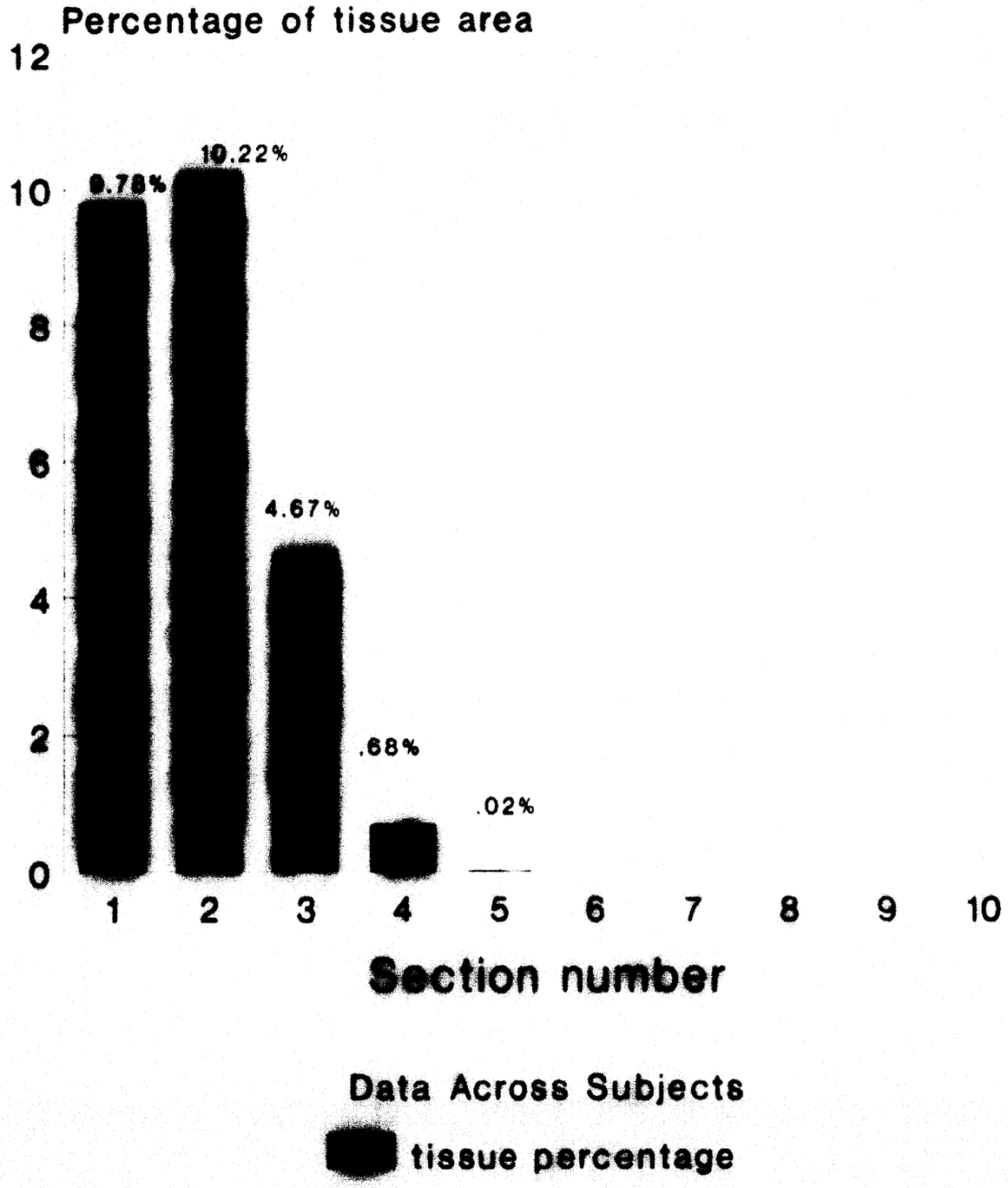


Figure 3.

GLANDULAR TISSUE
Area Ave./Percentages

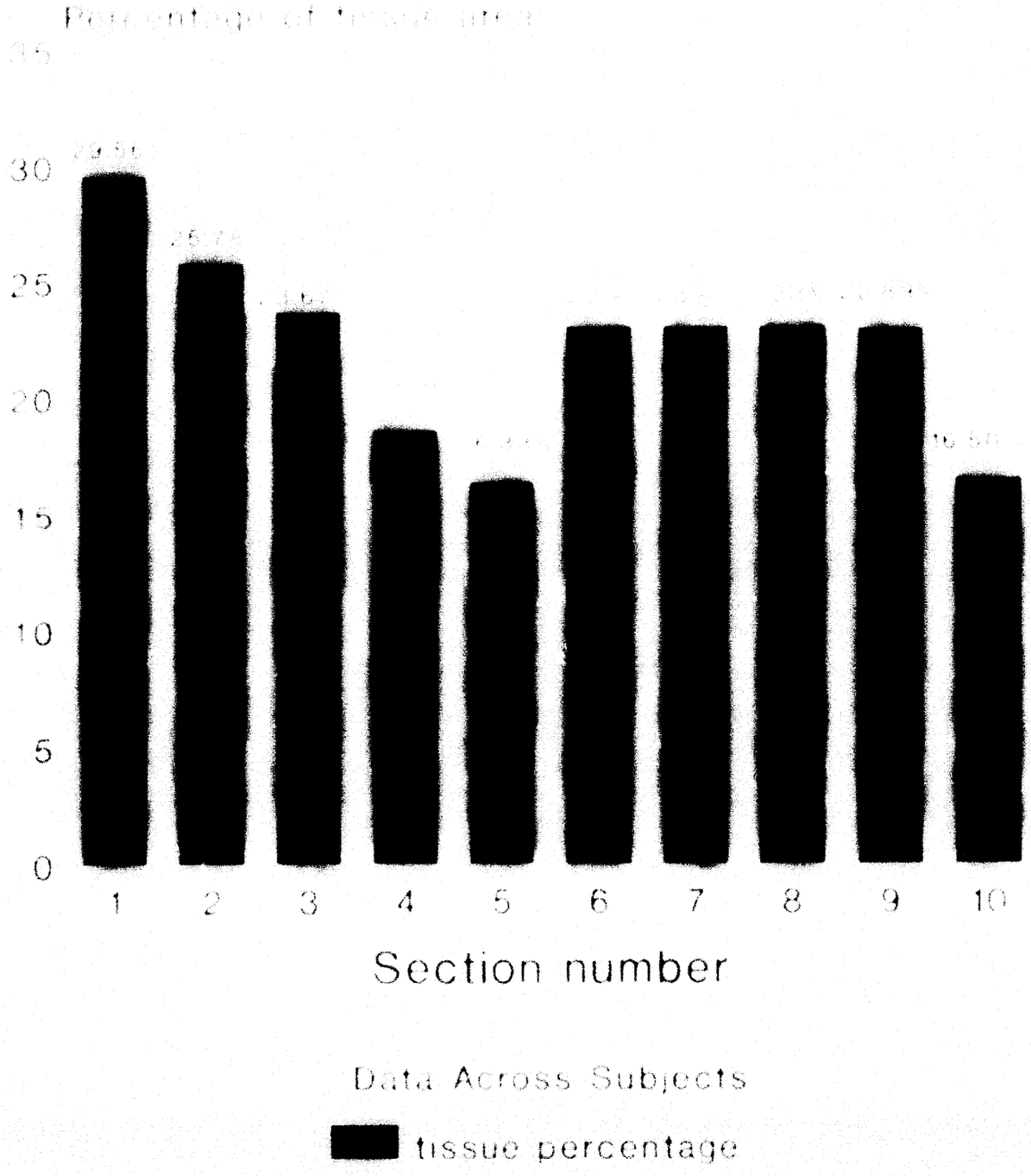
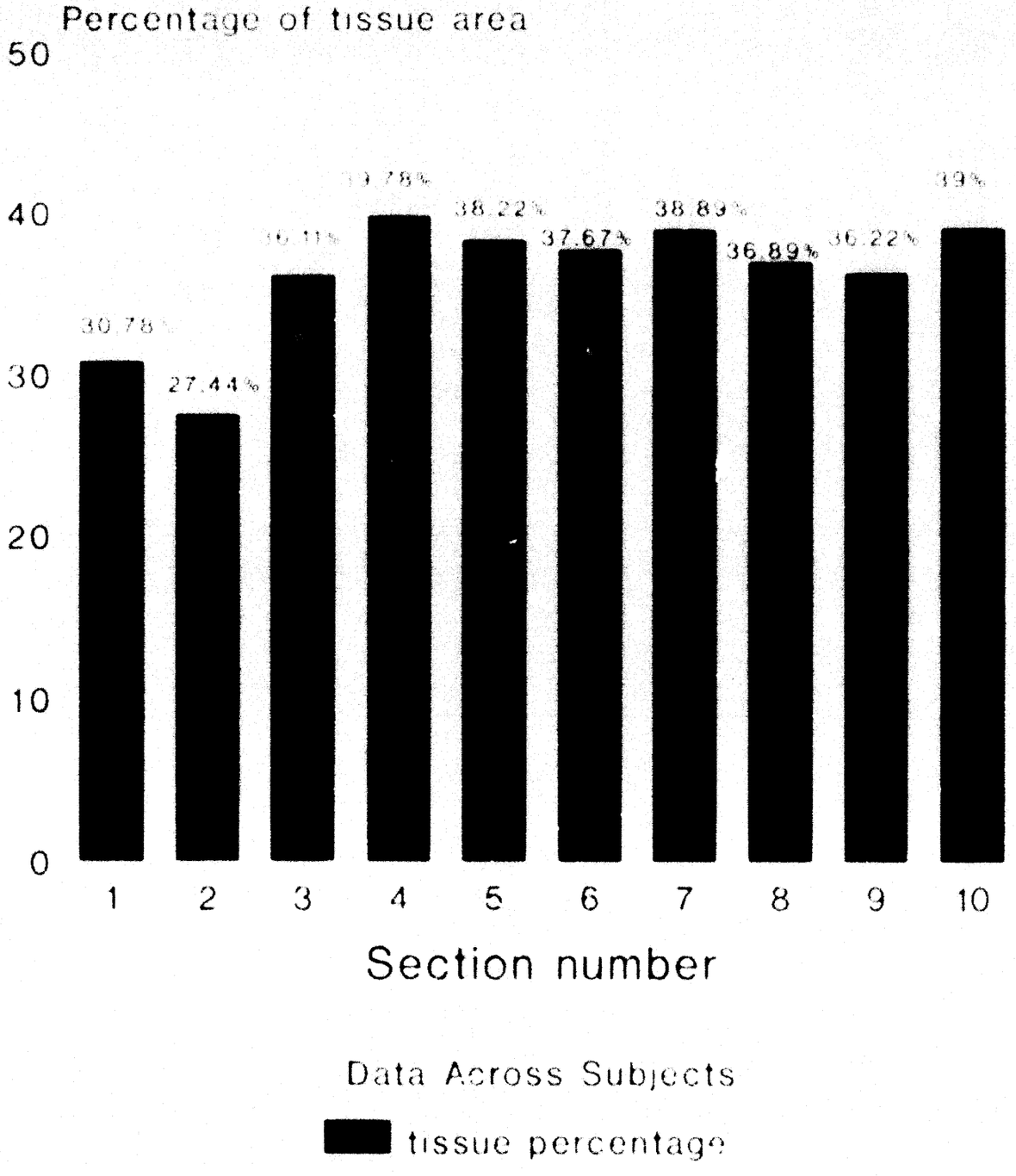


Figure 4.

CONNECTIVE TISSUE
Area Ave./Percentages



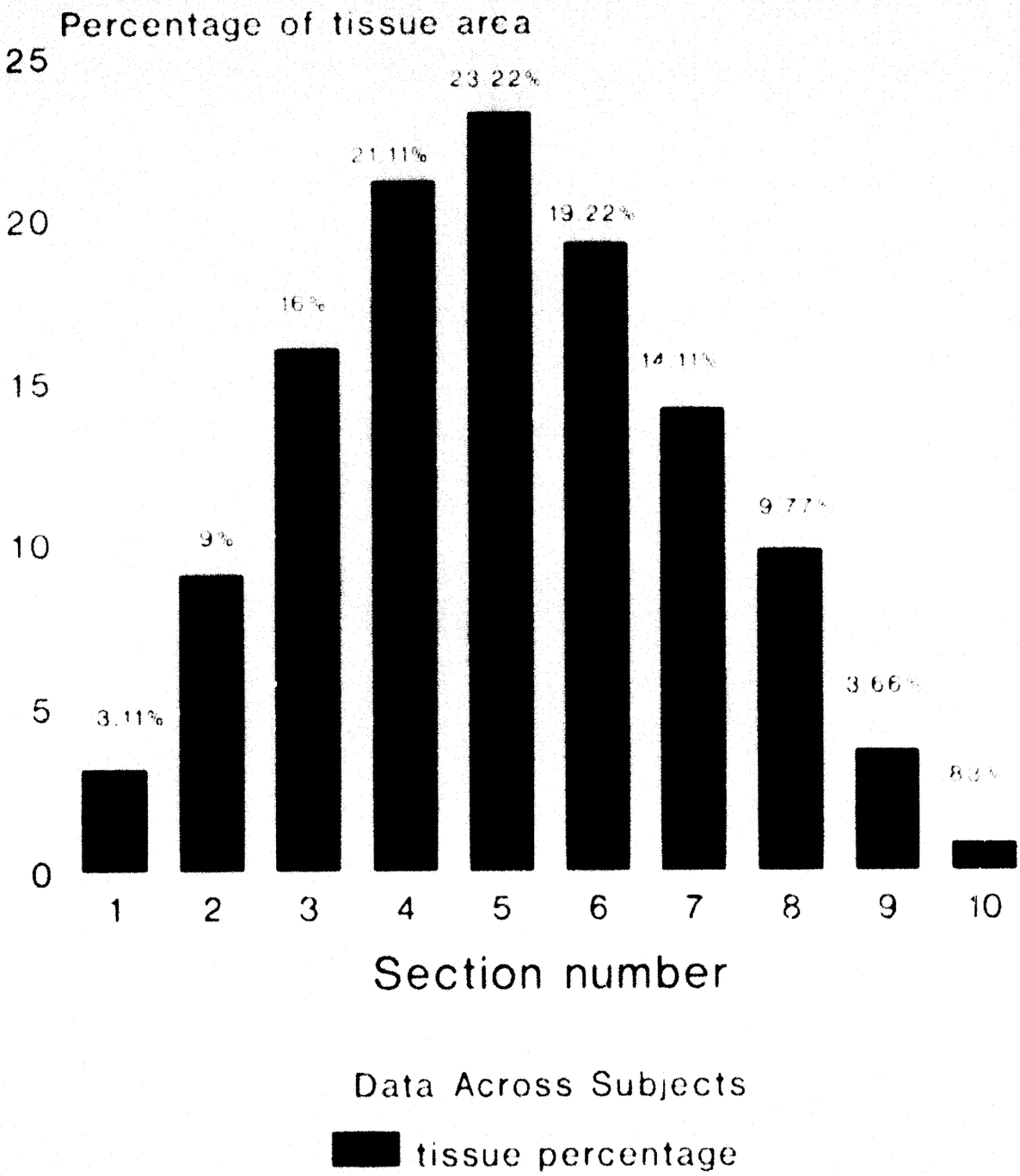
and Kahane (1990) and also that of Jesiolowski (1987). Percentages were not found across subjects and sections, however, in Jesiolowski's study, only within each subject and section. Comparisons, therefore, are understood as qualitative as is for the Kuehn and Kahane study. Glandular tissue seems to appear in lesser amounts than connective tissue, but compared to all other tissue compositions, both (glandular and connective) appear in the largest quantities. There are prominent areas of connective tissue beneath both the oral and nasal epithelium and less obvious areas intertwined throughout all sections in all directions.

Qualitative analysis, as compared to the two above mentioned studies previously conducted, show consistent findings. There is definitely a pattern of increasing muscle fibers, with the greatest quantity (about 23% of total tissue) found in section five. Decreasing muscle tissue amount continues until it is virtually absent in the final sections of the uvular base (Figure 5).

The distribution of adipose tissue seems to vary across all subjects and sections, which is consistent with Kuehn and Kahane (1990). Jesiolowski's (1987) reported that adipose tissue was found to increase

Figure 5.

MUSCULAR TISSUE
Area Ave./Percentages



along with muscle tissue, but this was not found in the present investigation. The adipose tissue seemed to remain at a constant level while muscle tissue increased. In fact, much more adipose tissue was found anteriorly (about 22% of total tissue) and then leveled off at a consistent level of about 17% of total tissue (Figure 6).

Other tissue, which consists primarily of epithelium and vascular tissue, showed a prominent increase in amount beginning with section seven. The other tissue remained constant throughout the first six sections and then increased to almost 30% of the total tissue at the uvular base (Figure 7). This was also found to be true in both the Kuehn and Kahane study and the Jesiolowski study.

When analyzing the data collected, results for absolute values as well as relative values normalized for the lateral boundary of the soft palate (J-Unit) were calculated. This was done to see if there was a significant difference between the two sets of data for each section and across all subjects. Only reports of a .5 mm^2 difference between absolute and relative

Figure 6.

ADIPOSE TISSUE
Area Ave./Percentages

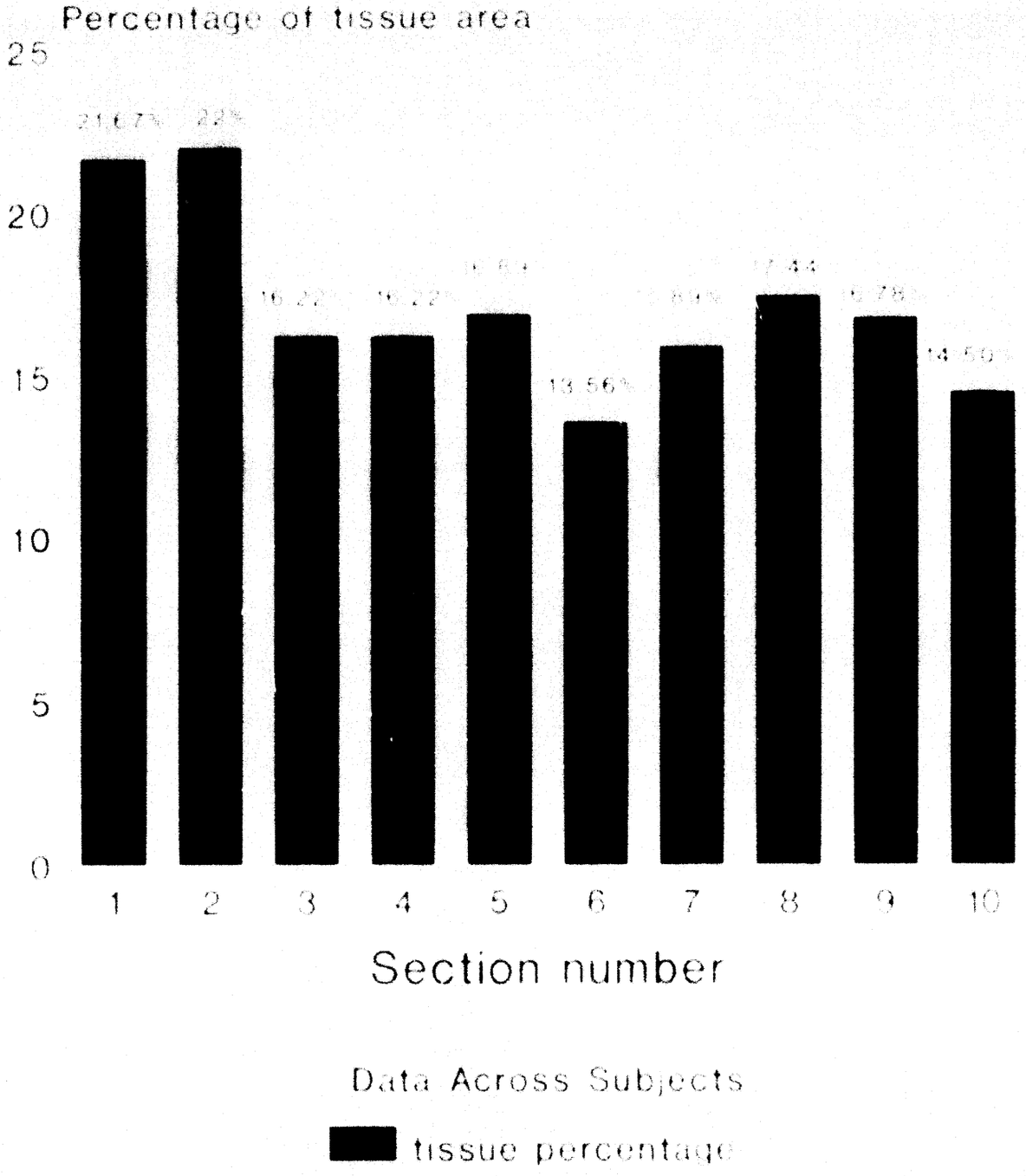
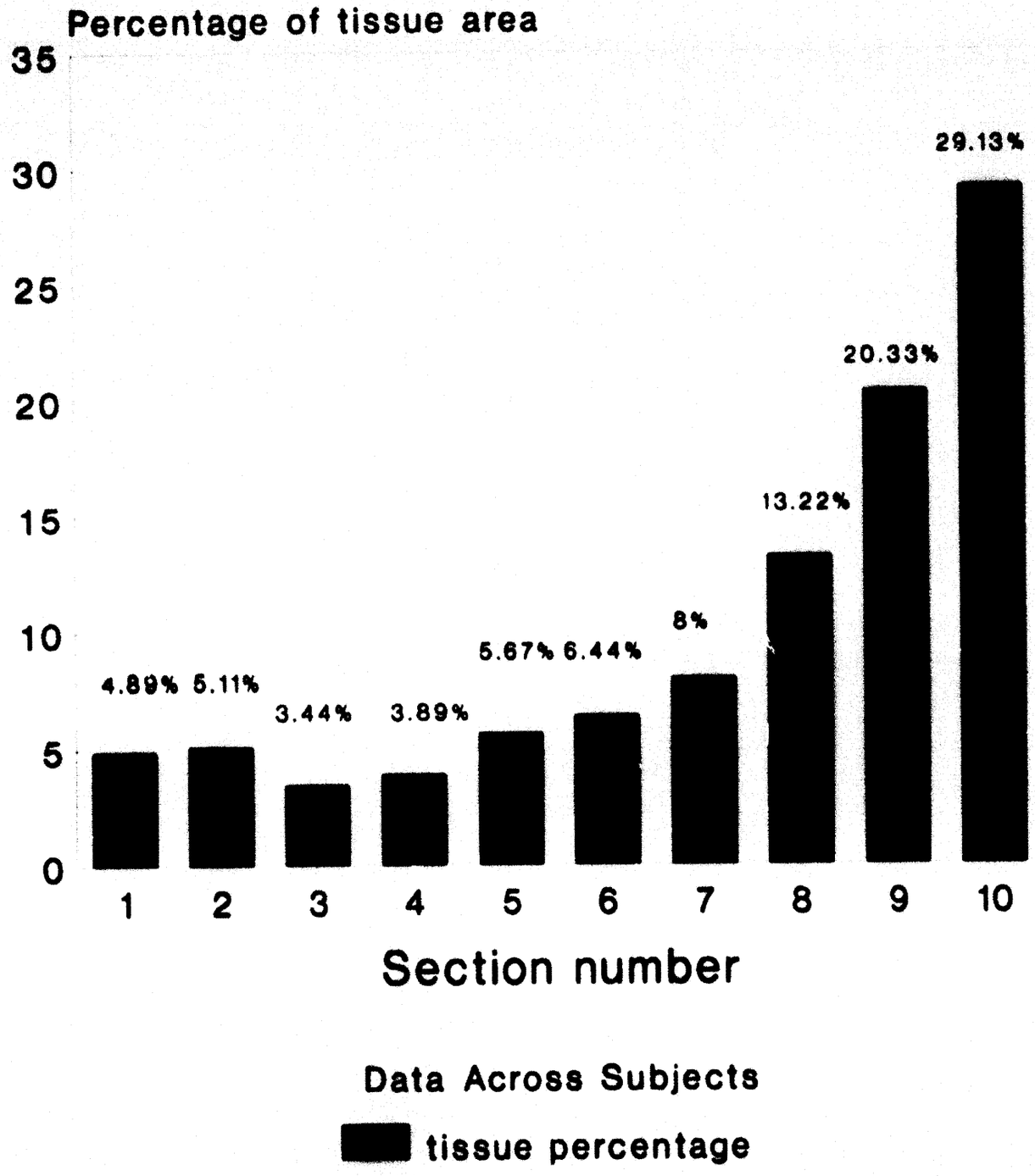


Figure 7.

OTHER TISSUE
Area Ave./Percentages



results will be noted because that seemed to be the largest difference on average.

Section one shows no differences between any of the tissues for absolute and relative values (Figure 8). Section two shows a .56 to .96 mm^2 difference range for glandular, connective, muscular, and adipose tissues which are among the larger differences between the absolute and relative values (Figure 9). Section three shows a .89 mm^2 difference in glandular tissue, which is the largest difference in this section among all the tissues (Figure 10). Section four depicts a range between .5 and .69 mm^2 difference for tendinous, glandular, and adipose tissues (Figure 11). It should be noted here that tendinous tissue was only found in a few subjects in section four. Section five only shows a difference greater than .5 mm^2 between the absolute and relative values in connective tissue (.58 mm^2 difference) and adipose tissue (.59 mm^2 difference) (Figure 12). It should also be noted here that both the analyzed values for tendinous tissue in this section were equal because only one subject displayed tendinous tissue in this section. Sections six through ten show no differences for absolute and relative

Figure 8.

SECTION 1

Area Ave./Absolute & Relative

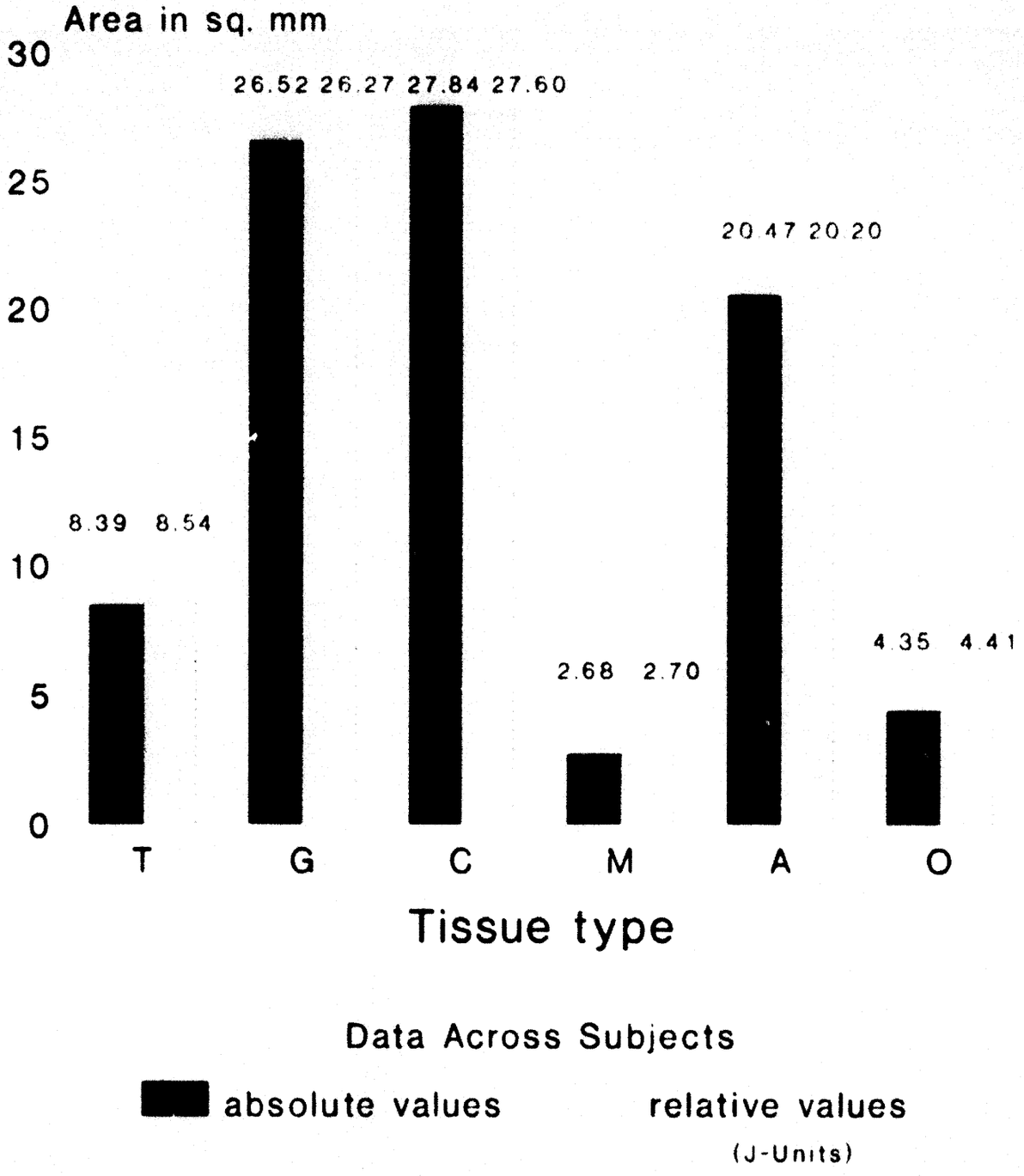


Figure 9.

SECTION 2

Area Ave./Absolute & Relative

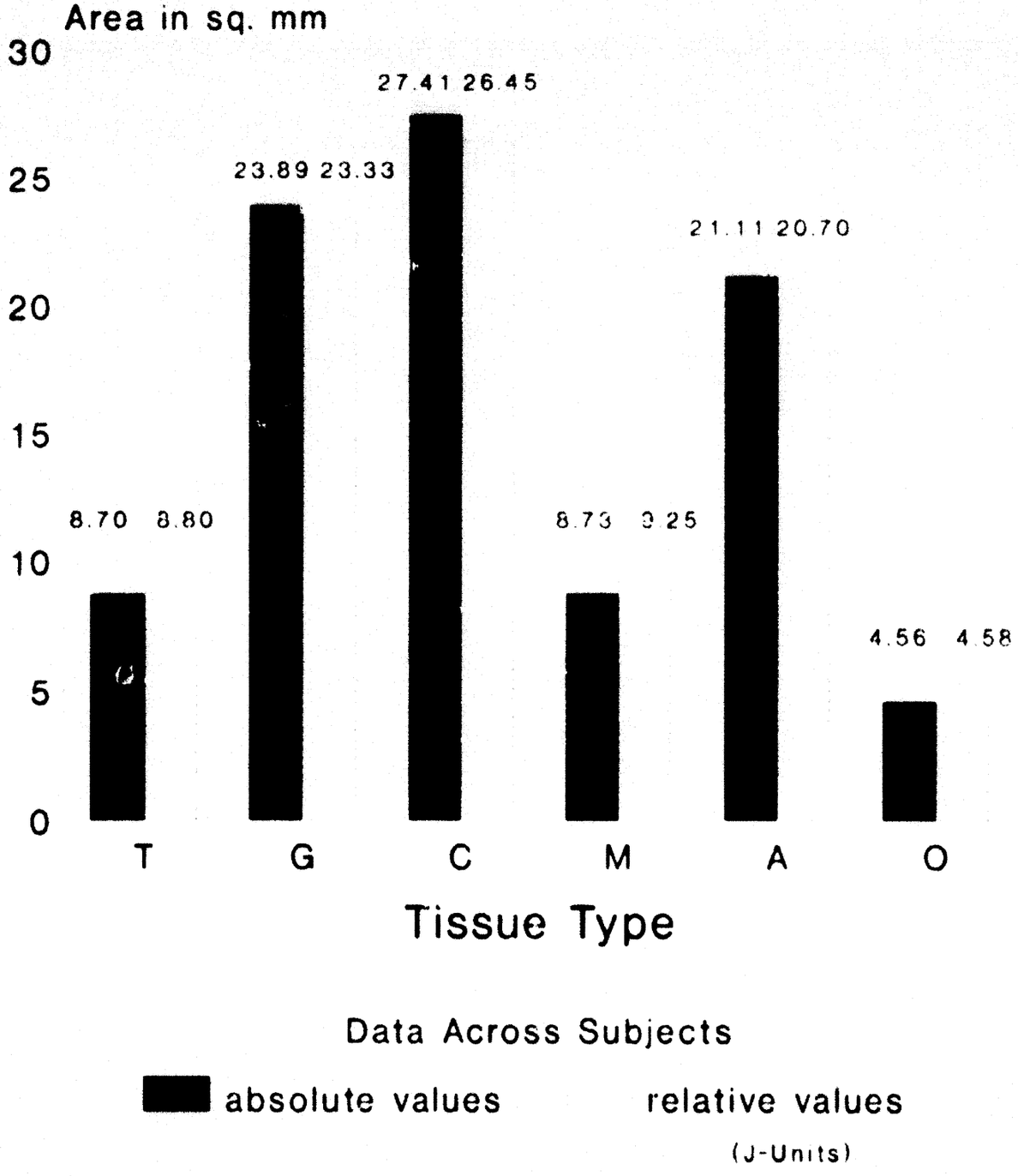


Figure 10.

SECTION 3

Area Ave./Absolute & Relative

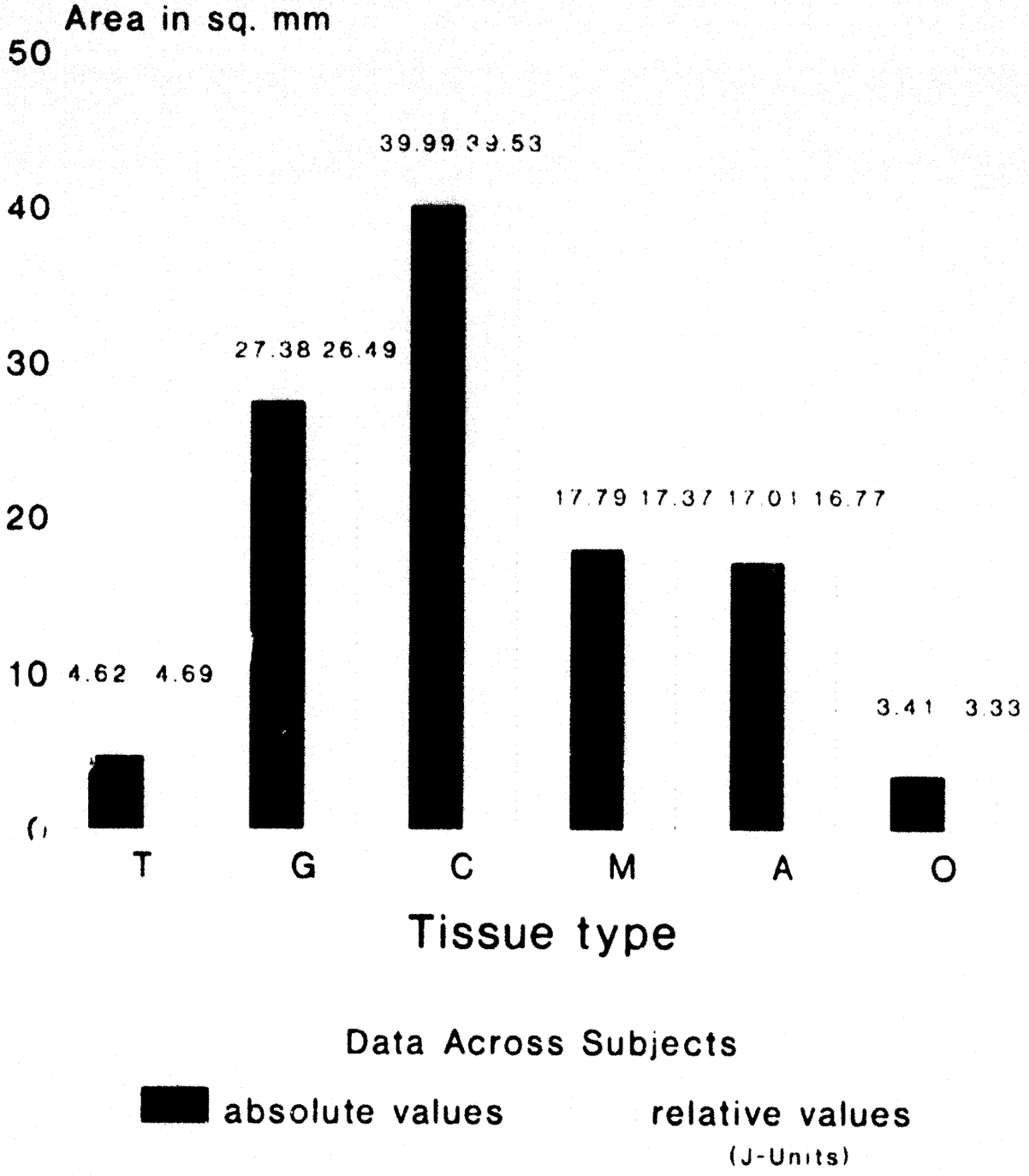


Figure 11.

SECTION 4
Area Ave./Absolute & Relative

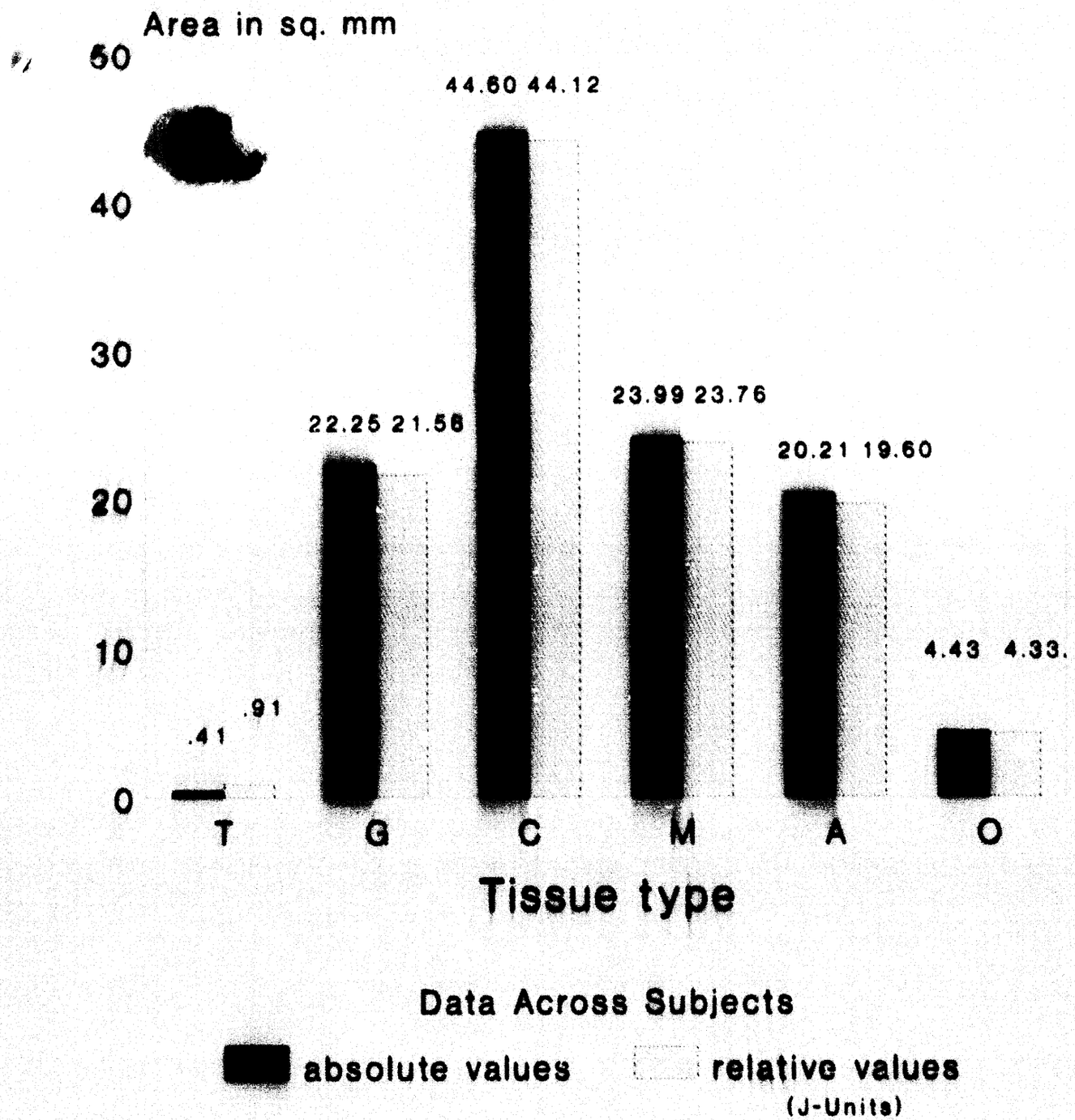
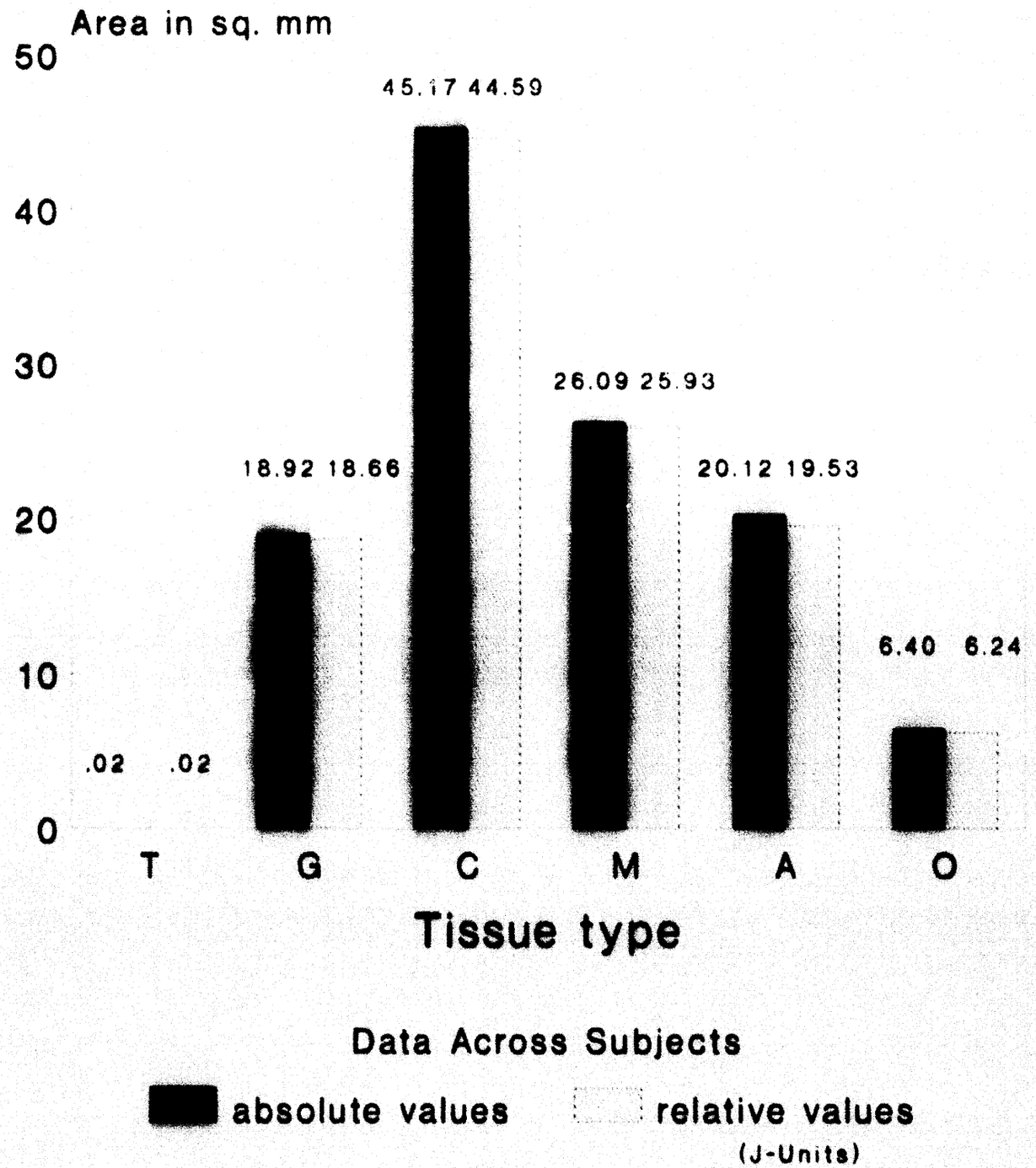


Figure 12.

SECTION 5

Area Ave./Absolute & Relative



values above .5 mm^2 for any tissue type (Figures 13-17). In fact, as one moves more posteriorly, the differences lessen and the values of area in mm^2 for both absolute and relative values are almost equal.

Figure 13.

SECTION 6

Area Ave./Absolute & Relative

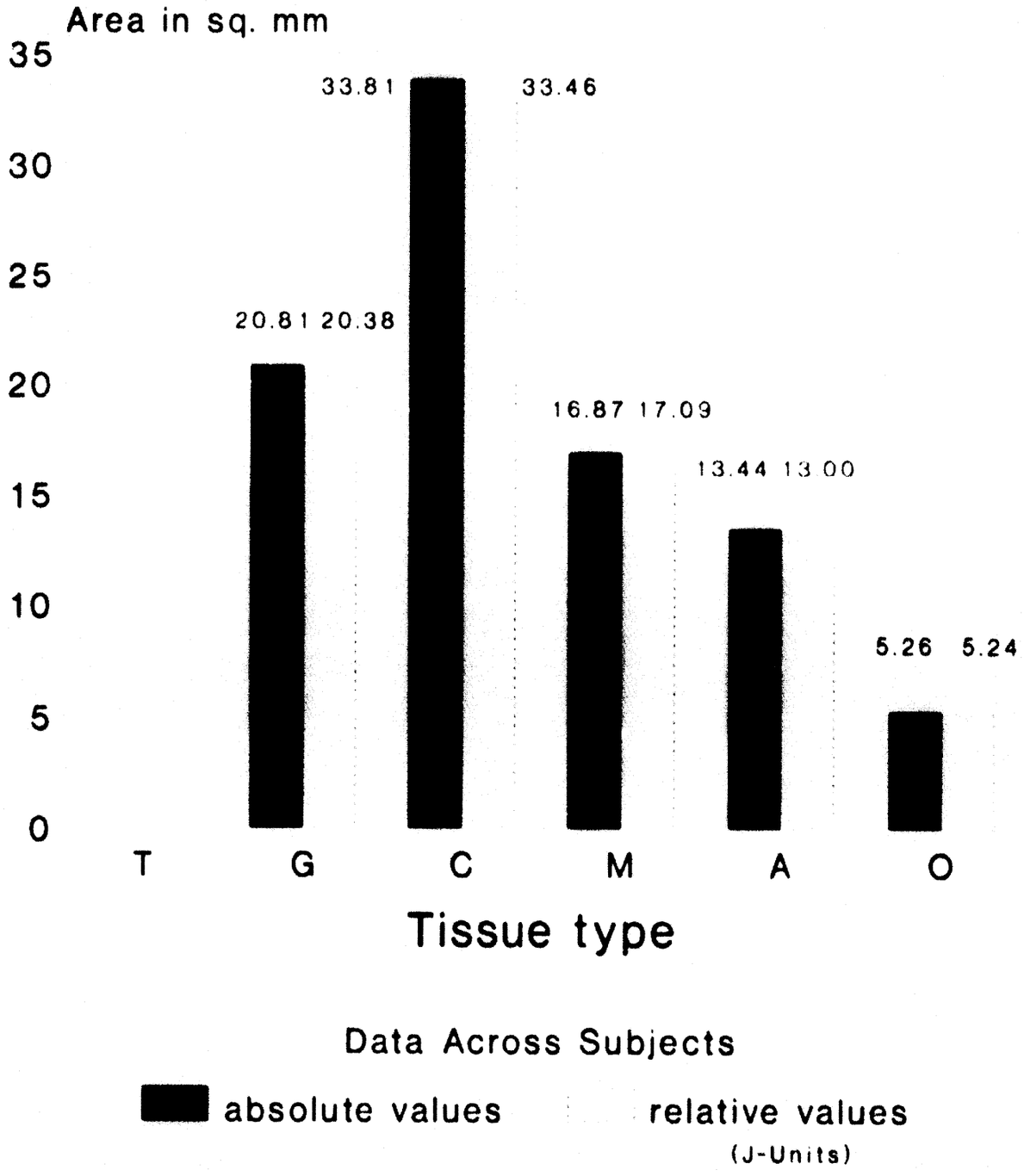


Figure 14.

SECTION 7

Area Ave./Absolute & Relative

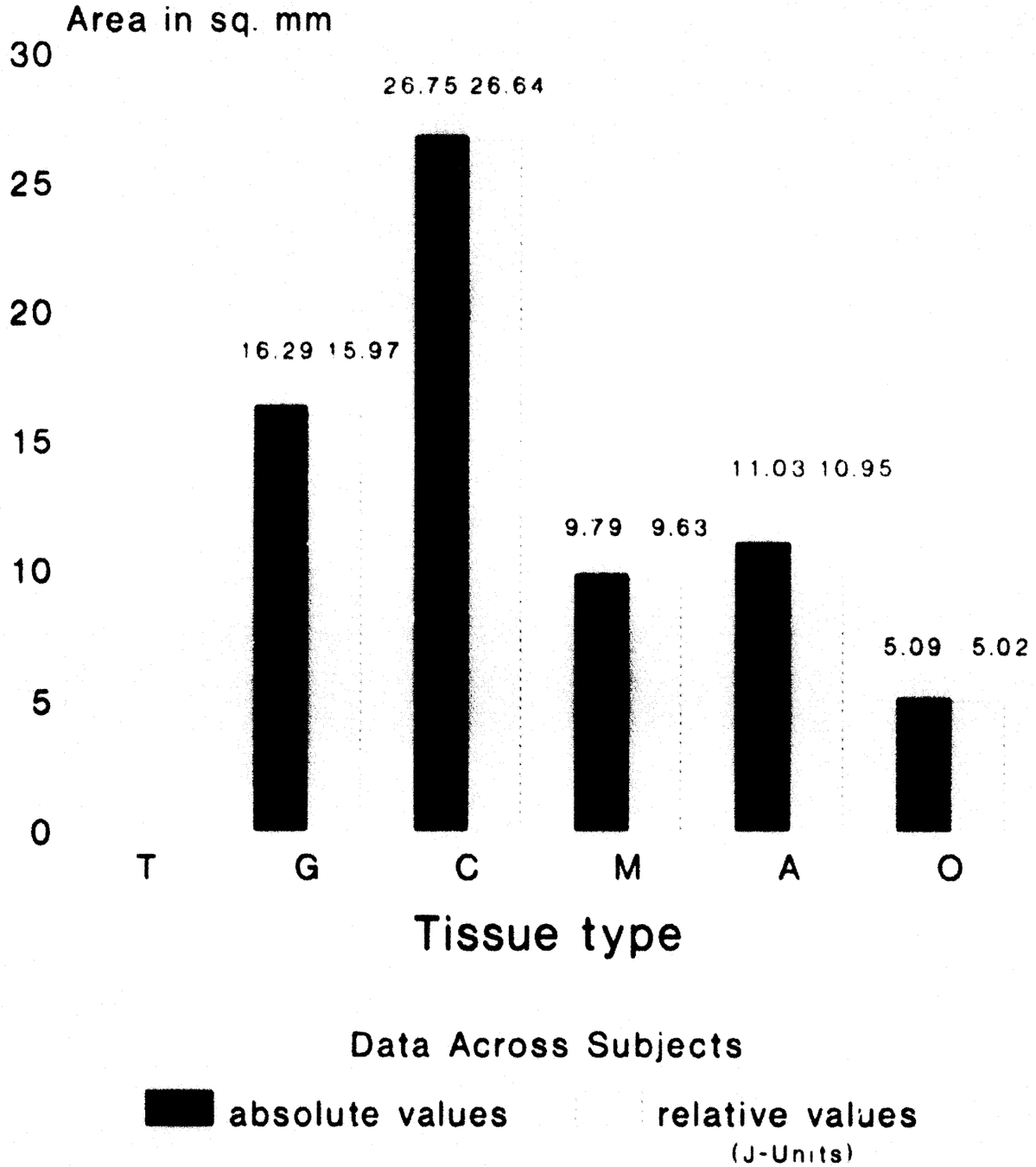


Figure 15.

SECTION 8

Area Ave./Absolute & Relative

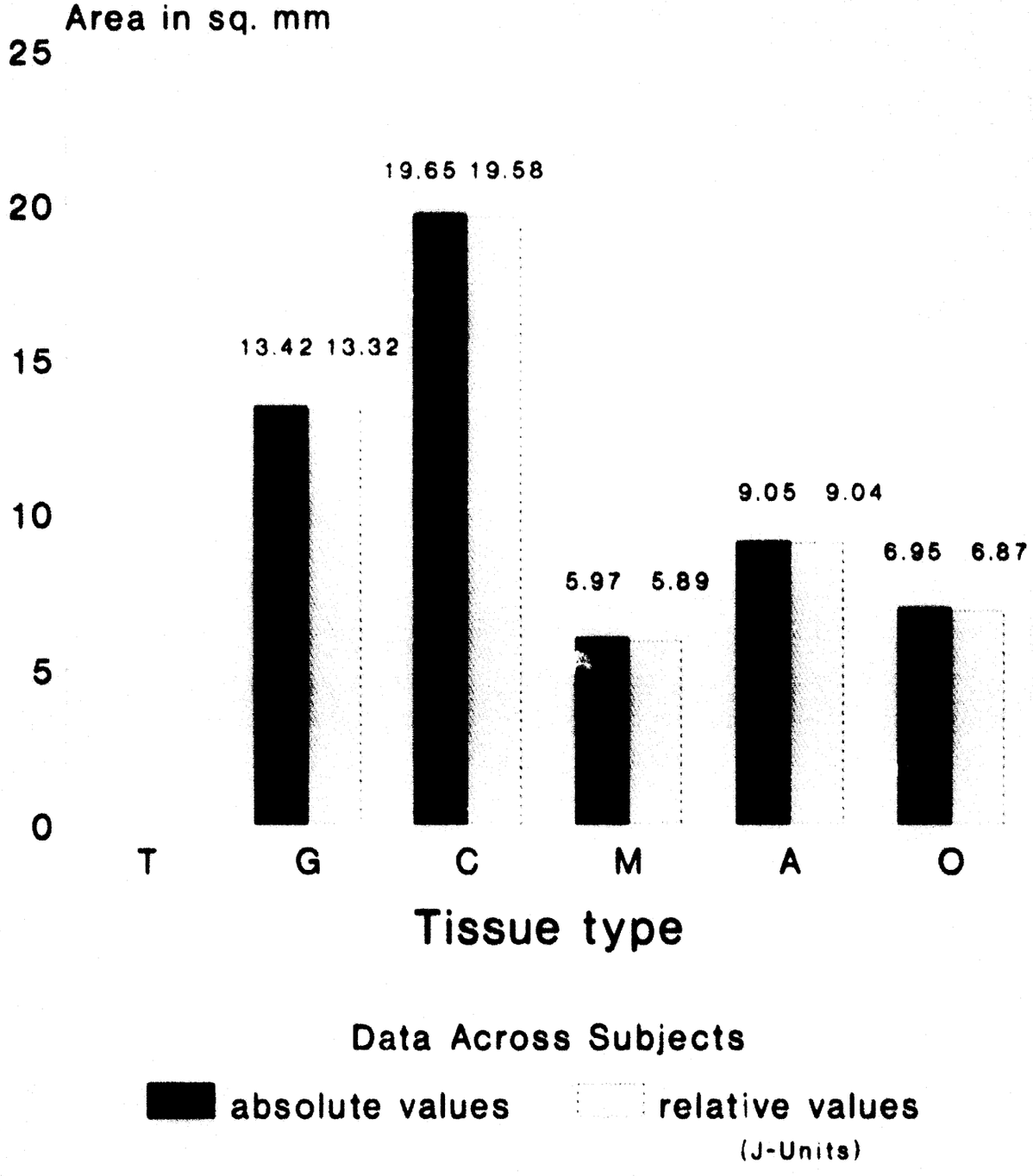


Figure 16.

SECTION 9

Area Ave./Absolute & Relative

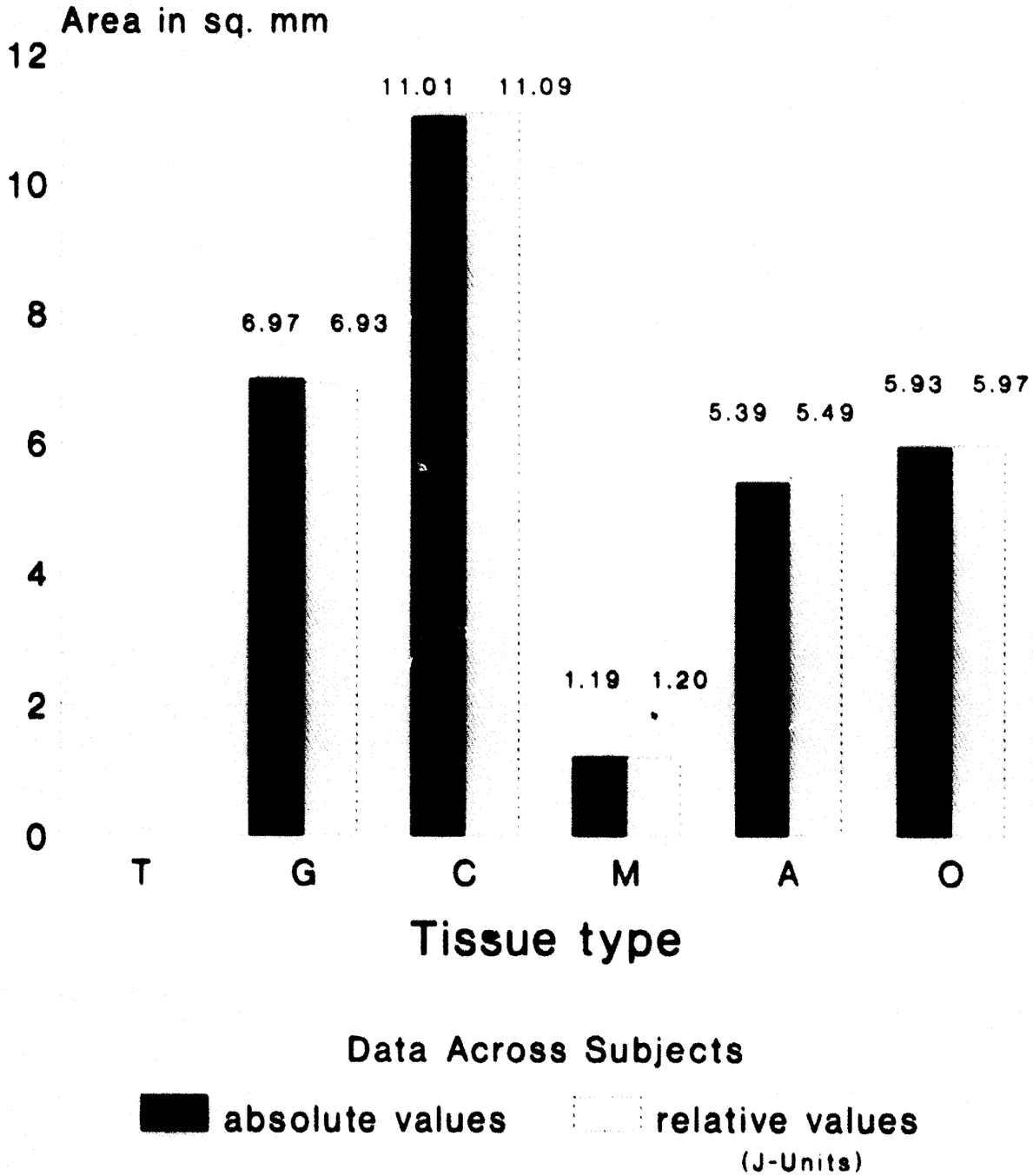
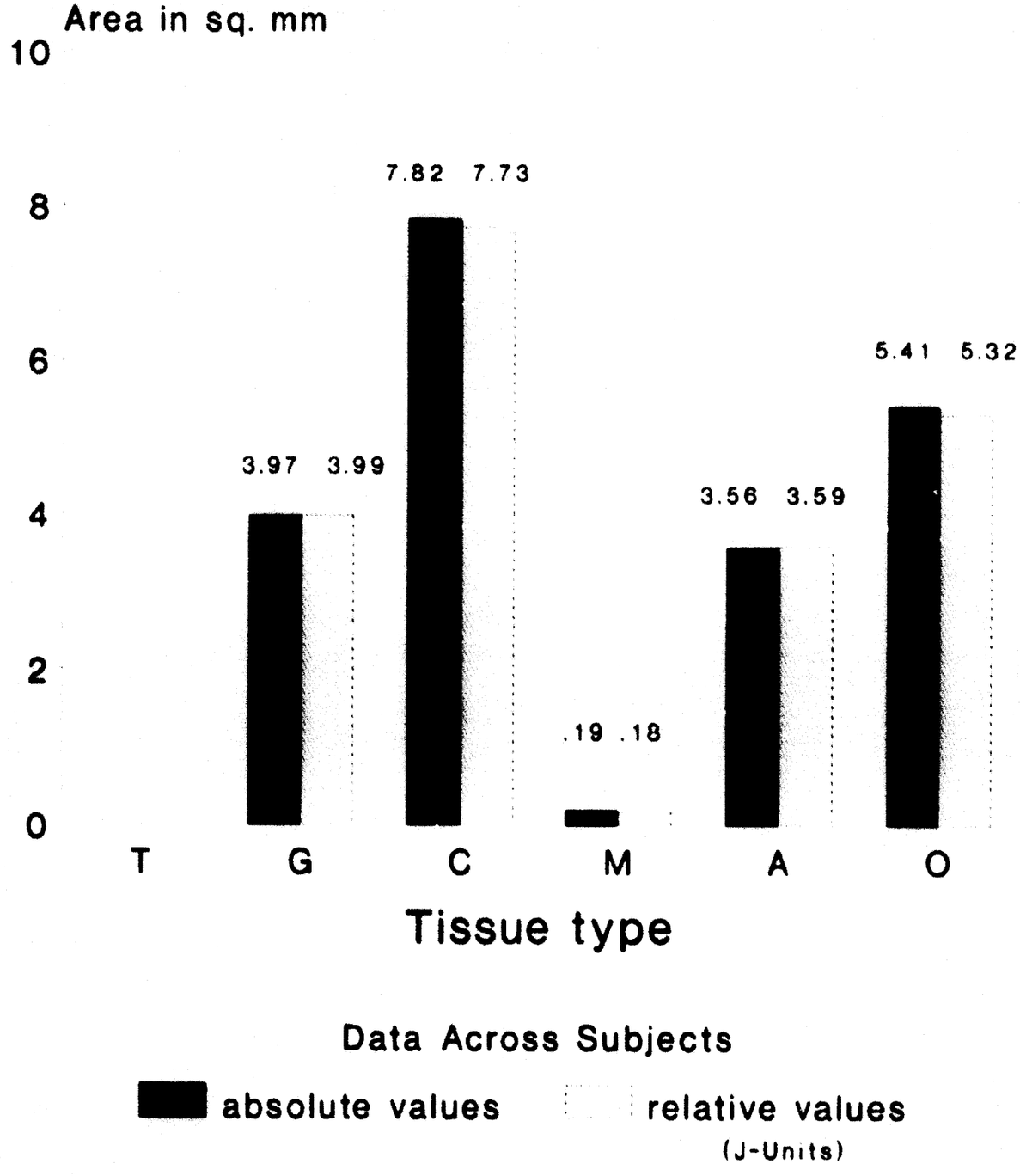


Figure 17.

SECTION 10

Area Ave./Absolute & Relative



CHAPTER V

DISCUSSION

Recent technologic advances in image analysis systems have made quantification of different tissue compositions much more feasible. The true color image analysis system used in the present study offers an important advantage over monochrome systems such as the Bioquant System IV used by Jesiolowski (1987). Specifically, with a multi-color stain such as the trichrome used in this study, it is much easier to identify and measure noncontiguous or fibrous tissue masses, which are much more difficult to visualize as separate distinct masses using a monochrome system. The Grains mode of the Videometric 150 is particularly advantageous in measuring noncontiguous or fibrous masses after they have been visualized in color.

The qualitative data reported in the Kuehn and

Kahane (1990) is consistent with the present investigation, however, quantified data have been recorded. These data, therefore, will be useful in developing a functional biomechanical model of the velopharyngeal mechanism.

Some aspects in the present study that could be changed if it were repeated would be to obtain a better light source. If one could improve the light source to a greater intensity, then the CCD camera could pick up more detail. Therefore, greater color differentiation of the different tissues (colors) and perhaps an increase in the accuracy of data collected would be achieved. This also leads into the problem of determining threshold for each tissue type (color). Determining threshold is a very subjective process. The experimenter could choose a different number of pixels (i.e. amount of color highlighting) for the same tissue type each time it was done. This subjectiveness could be lessened, however, if the light source was improved because color differentiation would, in turn, be greater and would lessen subjective judging of tissue type (color). Realizing the above problems, the present experimenter used the microscope as a cross-

validation method of the location and amount of tissue for each section and type of tissue. Viewing the slide under the microscope before collecting the data for each tissue type lessened the subjectiveness on the part of the experimenter.

Another problem encountered is the determination of the lateral aspects of the soft palate. As noted by the Kuehn and Kahane (1990), the soft palate is not a self-contained structure. The lateral boundaries of the soft palate blend into the lateral pharyngeal walls and are difficult to define. The lateral aspects of the soft palate were taken into consideration in the present study by use of the J-Units when collecting data. Also, obtaining relative values of area in mm^2 by normalizing the data to J-Units (i.e. making each subject's data larger or smaller as compared to the mean J-Unit value calculated) standardized the lateral boundaries of the velum. However, as noted by Kuehn and Kahane (1990), further investigation must be conducted to study extrinsic as well as intrinsic velar structures and relate them in a manner in which they might assist and/or limit the biomechanical characteristics of velar movements.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The main purpose of this study was to obtain quantitative results for the different tissue components in the normal human adult soft palate. This focus was taken because most of the current literature deals only with a qualitative analysis of the velopharyngeal mechanism. The collection of more objective measurements concerning the velum, therefore, will enhance our current knowledge and establish a firm foundation for further research dealing with the structure and function of the soft palate.

A thorough understanding of normal tissue distribution and quantification plays an important role in reconstructive surgical procedures. A review of the current literature is informative about the types of tissues located in different portions of the soft palate, but it does not evidence quantitative results

for the tissue components. Although this is important to first understand the basic structure of the velopharyngeal mechanism, more information and further research are needed to provide more detailed results of actual tissue quantity. This step was taken with the use of a monochrome image analysis system which has the ability to quantify specified areas of interest. This investigation was useful, but, unfortunately, it was never completed. Even more recent technologic advances in image analysis systems, however, have opened the door for even more accurate quantitative calculations.

For this study, a PC-Based true-color image analysis system was used to obtain quantitative results on one hundred individual histologic slides of bisected and proportionally sectioned soft palate specimens. From the results acquired in this study, a definite pattern of normal tissue distribution was noted as was consistent with previous investigations, but also quantitative measures were calculated. Tendinous tissue appeared on average as 3% of the total tissue area. Glandular and connective tissue components were the largest in composition reflecting 22% and 36% of the total tissue quantity on the average.

Approximately 14% was muscle tissue, and 18% appeared to be composed of adipose tissue. Other tissue components, consisting of epithelium and vascular tissue, appeared on the average as 7% of the total tissue area. Absolute and relative values, normalized by J-Unit (lateral boundary), also were calculated. Differences noted between the two pools of results were very small.

The results obtained in this investigation are another step further in the development of a functional biomechanical model of the velopharyngeal mechanism, but more information is still needed. Results of this study could have been more accurately gathered if an improved light source had been used, and the subjectivity was lessened in determining threshold luma (i.e. brightness) and specific colors (i.e. hue and saturation). Also further investigation must be conducted, as noted earlier, to determine how the extrinsic as well as intrinsic structures in the velopharyngeal mechanism affect velar movements. Even though normalized results were established according to the lateral boundary of the soft palate, more

information is needed to aid in the construction of biomechanical models.

The next step to be taken in the present investigation would be to obtain intra and interobserver reliability measures in which the former would reanalyze a subset of slides, and the latter would analyze a different subset of slide material. Then, perhaps, fiber density along with angulation could be calculated which would lead to an even more precise normal biomechanical model of a human adult soft palate.

With the results obtained in this study and with further investigation, it will be possible to process and display images taken with the use of magnetic resonance images (MRI) to construct a computerized three-dimensional model of the human velopharyngeal mechanism. The production of such a model will give rise to many advantages in the areas of basic research, clinical, and teaching applications. Basic research applications will involve the previously mentioned biomechanical models of the velopharyngeal mechanism which will provide useful information in speech, swallowing, and pathologic (cleft) investigations.

Clinical applications will involve computer simulations of cleft palate surgery which will allow one to "experiment" in a risk-free environment. Today, information concerning the anatomy and physiology of the speech and velopharyngeal mechanisms is given exclusively by means of lecture and/or demonstration. This form of presentation makes the material difficult to absorb. However, if the student had access to a computer with three-dimensional and rotational features of structures and color-coded muscle masses, this problem of "lecturing" could be overcome. The student in this way would have a rich environment in which to explore the material directly and view it from any perspective. All of the above mentioned could be achieved if one would just make the step in the next direction to follow up the present investigation.

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