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The importance of humidity in the *in vitro* study of the cranium with regard to initial bone displacement after force application

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SUMMARY The aim of this pilot study was to find a suitable model for simulating *in vivo* initial displacements of the maxilla after force application. Force was exerted on the maxillae of four dogs by means of a dental splint. To register the bone displacement, speckle interferometry was used as the measurement technique. The initial orthopaedic effect as a result of force application *in vivo* was registered by the construction of rotation centres around which the maxilla rotated. The orthopaedic effect after force application *in vivo* was compared with different *in vitro* situations of the laboratory animal. After the animal had been killed and its maxilla macerated, the cranium was placed in a sealed conditioned box. Only three skulls were found to be suitable for this experiment. The environment thus created can be influenced by changes in relative humidity. Various forces were applied in various directions on the maxilla of three Alsatians. By changing the relative humidity, an attempt was made to create a situation in which the initial bone displacements after force application were comparable to the *in vivo* situation. None of the laboratory animals used showed a comparable condition for the *in vivo* situation through manipulation of relative humidity. As only three dogs were experimented on, these findings are tentative. This experiment will be extended to three more dogs from the same litter.

Introduction

Dento-maxillo-facial orthopaedics in orthodontics have been introduced in an attempt to influence the development and growth of the maxilla and mandible. Orthopaedic appliances have been developed and their clinical effects on groups of patients have been investigated in order to understand the underlying mechanism of their activity. One appliance can have a different effect on different patients due to variations in growth patterns and cranium morphology. However, the testing of different orthopaedic instruments on just one patient is excluded, if only for ethical reasons. It is therefore obvious that a 'model' needs to be found, all individual parameters of which can be kept constant and on which various orthopaedic appliances can be tested. A proper model could be suitable to test the working or development of new types of orthodontic appliances, presuming that the link exists between initial bone displacement after force application on the *in vitro* model and the possible effect on growth in comparable circumstances. The 'dry cranium model' could be suitable to this end. Although this model has a number of limitations compared with the *in vivo* situation, it can be argued that this macerated morphological model significantly approaches a number of qualities of a living laboratory animal. The initial bone displacements immediately after force application could be an indication of secondary bone remodelling processes induced by this appliance in the long term.

Several studies have described the initial displacements caused by extra oral forces on teeth and bone structures of the cranium (Fryman, 1971; Ichikawa *et al.*, 1984; Dermaut *et al.*, 1986, 1992). Cranial deformations after force application induce traction and compression areas in the sutures. Previous studies have used mainly strain gauges on clean and dry bone

surfaces to measure bone deformation (Miura *et al.*, 1980; Kannan, 1982; Ichikawa *et al.*, 1984). 'Double exposure holography' has also been used as a non-destructive testing device to measure initial displacements (Nanda, 1978; Burstone and Pryputniewicz, 1980; Dermaut and Beerden, 1981; Van den Bulcke *et al.*, 1986), as has laser speckle interferometry (Kragt, 1979; Pryputniewicz *et al.*, 1980; Kleutgen *et al.*, 1982; Kragt and Duterloo, 1982, 1983; Kragt *et al.*, 1982, 1984; Dermaut *et al.*, 1986; De Clerck *et al.*, 1990). The value of the macerated skull as a model used in orthopaedic research has been tested by De Clerck *et al.* (1990). They showed that the 'dry macerated cranium' (as an *in vitro* model) was not a suitable model to simulate initial bone displacements (*in vivo*) after anterior traction on the maxilla. The centres of rotation of the maxilla after force application *in vivo* were not found to be an indication of the localization of those found after initial displacement of the cranium. After being moistened, the dry cranium seemed to approach the *in vivo* situation in certain circumstances. The aim of the present study was to examine whether manipulation of relative humidity (RH) could result in the creation of the ideal model for *in vitro* research. RH is the amount of moisture in the air as compared with the amount that the air could contain at the same temperature, expressed as a percentage.

Materials and methods

Materials

Initially four female Alsatians, all 11 months old, were used in this study. Although the dry cranium model after maceration has many limitations compared with the *in vivo* situation, it can be argued that this macerated morphologic model significantly approaches a number of properties of the living animal. Cranial size, cranial morphology appropriate fixation, manageability and availability of this type of dog make the cranium model suitable for this study. The anterior development of the maxilla in dogs is morphologically favourable for the measurement of displacements after anterior force application.

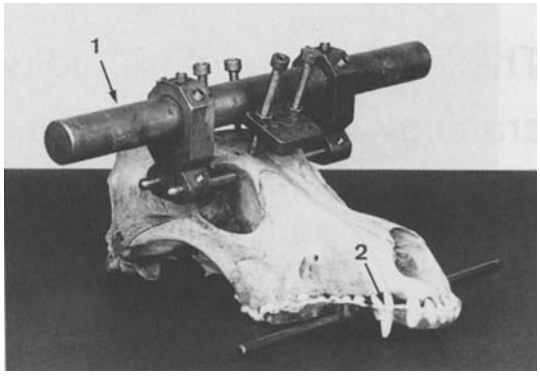

Figure 1 The frontal clamp (1) developed for fixation *in vivo* and *in vitro* with the acrylic splint (2) fixed to the upper dental arch.

Methods

The displacement of the maxilla, no larger than a few microns, was measured with reference to the frontal bone. Initial displacements *in vitro* as well as *in vivo* through force application on the maxilla were measured by means of 'speckle interferometry' (Pryputniewicz *et al.*, 1980). With this technique there is no direct contact between bone structure and measuring equipment. In order to evaluate the different displacements of the maxilla for both registrations a frontal clamp (Figure 1) was used. Initially the head of the living animal was fixed into the clamp. The clamp and head were then fixed onto an L-shaped stand (Figure 2). This fixation system was introduced and first tested by De Clerck *et al.* (1990). To enable the measurement of the displacement *in vivo*, a metal wire, with a measuring plate at one end, was fixed with a screw to the bone surface. A second measuring plate was fixed in the same way onto the posterior part of the zygomatic arch to allow control of the stability of the clamp (Figure 3). By means of a laser beam directed towards these plates, a speckle pattern was registered on a photographic plate. After displacement of the maxilla (due to force application) a second exposure was registered on top of the first. The registered 'double exposed specklegram' permitted the construction of the centre of rotation around which the maxilla initially rotates. The displacement vector could be

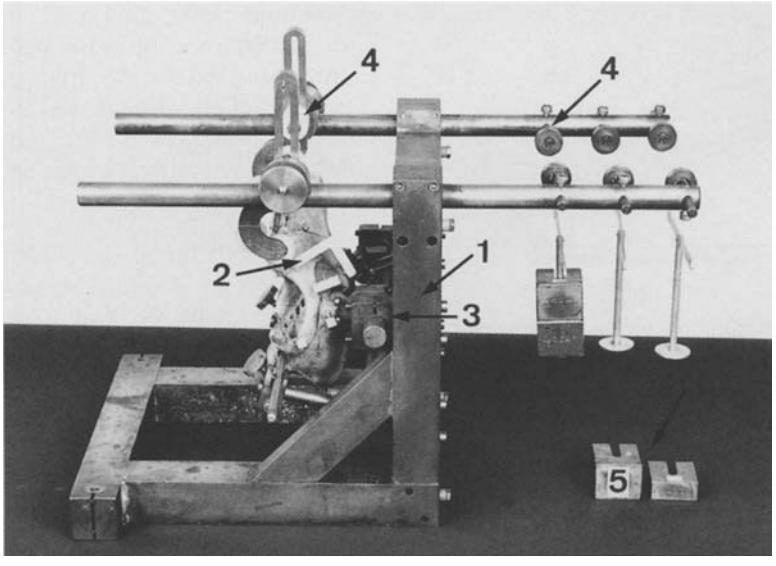

Figure 2 The L-shaped stand (1) with measuring plates on bone (2) and frontal clamp (3), the pulleys (4) for different traction directions and different weights (5) for various amounts of force.

reproduced at three different points on the rectangular measuring plate. The centre of rotation was constructed by the intersection of the lines starting at each of the three measuring points, perpendicular to the displacement vectors. Although two points were sufficient, a third one was used to test the reliability (all the vectors should intersect through the same point). Consequently, the orthopaedic effect could be studied for each traction by means of the location of the centre of rotation and the amount of displacement.

At the end of the *in vivo* experiments, the animals were killed and the heads of the animals macerated post-mortem using African beetles. After maceration was completed, the cranium with frontal clamp and the measuring plates were then repositioned for the *in vitro* registrations exactly in the same way as *in vivo*.

In the first part of the study, *in vivo* measurements were carried out on the animals under general anaesthesia. Later, after killing, the same procedure was repeated on the craniums of the same animals.

Thus, both centres of rotation could be compared in an attempt to test the value of the skull as a model for orthopaedic research after initial displacements.

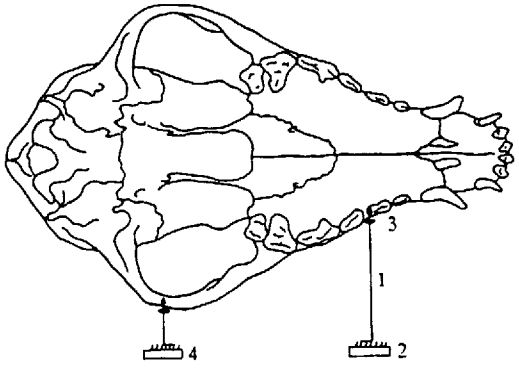

Figure 3 The measuring plates *in vivo* and *in vitro*: the metal wire (1) with the measuring plate (2) screwed to the bone surface of the maxilla (3) and the zygomatic arch (4).

A system consisting of pulleys was designed to standardize the different tractions (Figure 4). Nylon threads ran from one side of the dental splint over various pulleys to a bar on the other side. Weights influenced the amount of force on the dental splint and therefore on the maxilla. The in-between position of the various pulleys allowed for high-pull, ventral and cervical tractions on the maxilla. Thus it was possible to register maxillary displacements in different directions.

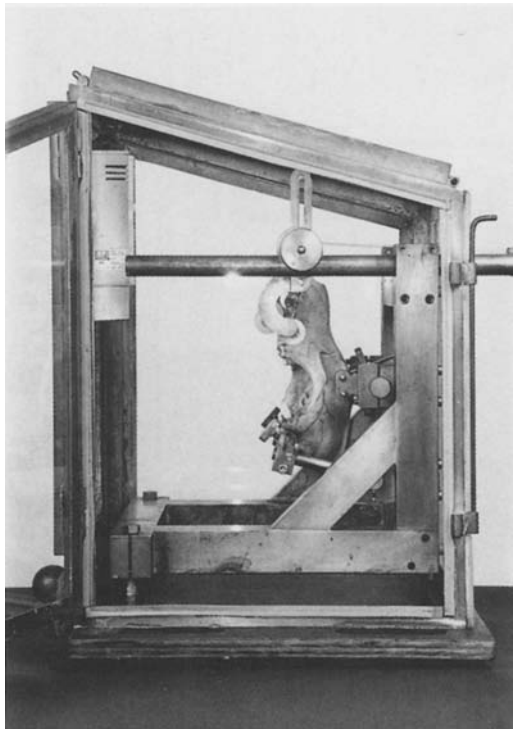

Figure 4 The sealed conditioning box for *in vitro* registrations.

A sealed conditioned box for the *in vitro* registrations was constructed in order to be able to condition the cranium to a certain humidity (Figure 4). Conditioning of the cranium can be effected in a predefined environment with preset temperature and relative humidity. Measurements took place at a temperature of 24°C at relative humidity values of 40, 60, 80 and 100 per cent. Photographic registrations took place *in vitro* through the glass housing of the sealed conditioned box. A heating element with a sensor kept the temperature at a constant value. Salts were diluted with water to achieve a constant air humidity level, the solution covering the bottom of the box but not being in direct contact with the cranium.

Forces were applied in various sequences to neutralize the previous direction of traction. Every direction of traction was repeated ten times, but after five tractions a different direction was applied. The forces applied were 450, 600, 800 and 1100 g. Loads in three different directions were applied *in vivo* to four Alsatians, the direction being high-pull, ventral and cervical. One animal was excluded from any further testing of the cranium since the system fell apart after maceration due to poor fixation.

Error of the method

The error of the method has been tested by De Clerck *et al.* (1990). Measurement errors of approximately 1°C and 1 μm were found for displacements smaller than 10 μm. In none of the Alsatians could any reliable displacement caused by high-pull traction be measured at any force level applied, either in the *in vivo* or in the *in vitro* experiments. The same was found for all measurements carried out at 40 per cent RH. Therefore these measurements were not carried out in this study. For the other displacements, the measuring error (1 μm for displacements larger than 10 μm) was smaller than 10 per cent.

Results

In one dog (dog A) the effect of temperature was tested. Both ventral and cervical traction was executed at temperatures of 24 and 34°C. Each measurement was carried out 20 times at 100 per cent RH. Figure 5 shows the location of the average values for both traction directions. The average value for the centre of rotation in the case of ventral traction was higher and more forward than in the cervical one. However, the average values for the same traction direction for both temperatures were located very close to each other. The amount of force application ranged between 450 and 1100 g. Five loadings were applied to each dog for each traction direction. No significant changes in the location of the centres of rotation were found as a result of changes in either force level or temperature. Moreover, this procedure turned out to be very difficult: maintaining the humidity level at two different temperatures was critical in the experimental design used. Therefore the effect of temperature was not incorporated in the other experiments.

In Figure 6 the centres of rotation after ventral traction for one dog (dog A) are plotted for the *in vivo* situation and at different humidity levels.

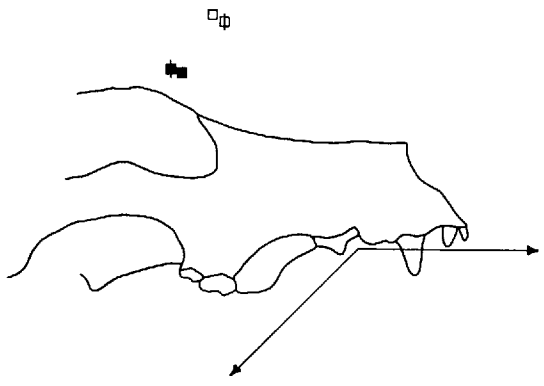

Figure 5 The average values of the centres of rotation for ventral (□, ⊞) and cervical (■, ⊞) traction directions at different temperatures of 24°C (□ , ■) and 34°C (⊞, ⊞) in dog A at 100 per cent RH.

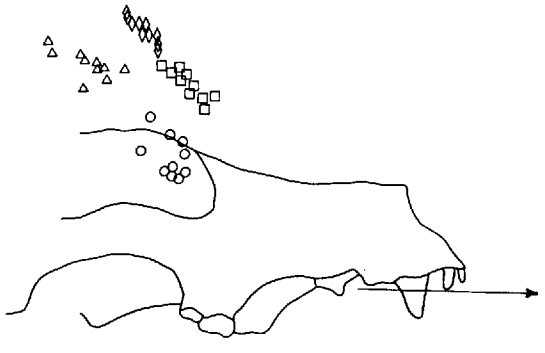

Figure 6 Clustering of the centres of rotation after ventral traction for one dog (dog A) are plotted for the *in vivo* situation (○) and at humidity levels of 100 (□), 80 (◇) and 60 (△) per cent RH.

The same procedure was followed for the other skulls *in vivo* as well as *in vitro* and in cervical traction. In dog A the centres of rotation cluster *in vivo* at the orbit and the clouds of points wave upwards and backwards between 100 and 60 per cent RH. Each measurement was carried out 10 times.

Experimental animal A: in vivo and in vitro (Figure 7)

The results from all the parameters tested after both ventral and cervical tractions were

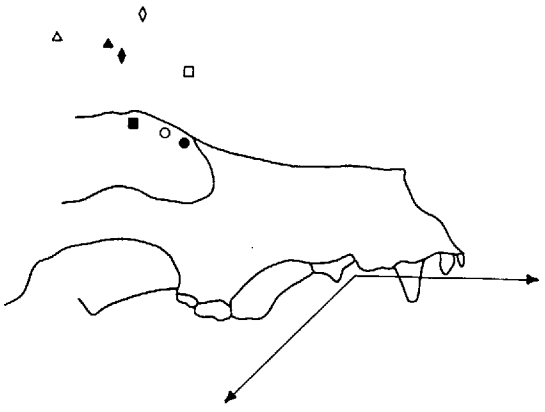

Figure 7 The average values of the centres of rotation after ventral (open symbols) and cervical (closed symbols) tractions in dog A for the in vivo situation (○, ●) and at humidity levels of 100 (□, ■), 80 (◇, ◆) and 60 (△, ▲) per cent RH.

compiled for dog A. The average value of the centres of rotation was measured for the *in vivo* situation and at different humidity levels (100, 80 and 60 per cent RH). Ventral and cervical average values for the centres of rotation *in vivo* were concentrated closely and were both located in the orbit. The ventral average value of the centre was situated a little more backwards and upwards. The cervical average value at 100 per cent RH was found further towards the back and a little higher than was the case in the *in vivo* situation; however, it was still located within the orbit. The ventral average value was situated higher and located more forward outside the orbit than in the *in vivo* situation. When the cranium was dried further (registrations taken at 80 per cent RH) the average values of the centres of rotation for both the ventral and cervical tractions were found to be higher and more backwards. The cervical average value at 60 per cent almost coincided with the average value at 80 per cent. The ventral average value was found more backwards and lower when compared with the values at 80 per cent RH. Moreover, the points of 100 per cent RH were situated closest to the *in vivo* points.

Experimental animal B: in vivo and in vitro (Figure 8)

In dog B the average value of ventral tractions

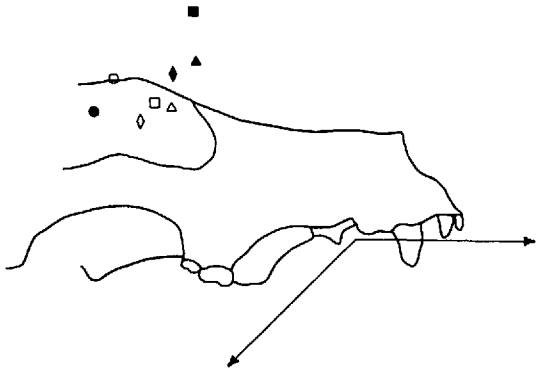

Figure 8 The average values of the centres of rotation after ventral (open symbols) and cervical (closed symbols) tractions in dog B for the *in vivo* situation (○, ●) and at humidity levels of 100 (□, ■), 80 (◇, ◆) and 60 (△, ▲) per cent RH.

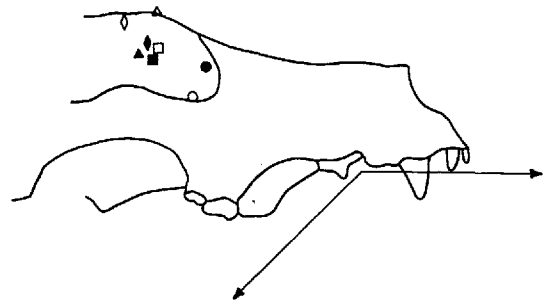

Figure 9 The average values of the centres of rotation after ventral (open symbols) and cervical (closed symbols) tractions in dog C for the *in vivo* situation (○, ●) and at humidity levels of 100 (□, ■), 80 (◇, ◆) and 60 (△, ▲) per cent RH.

applied *in vivo* was found at the back on the edge of the orbit. The average value of cervical traction was found more dorsal and lower than the ventral average value. The registrations taken at 100 per cent RH showed a ventral average value situated more towards the middle of the orbit and somewhat lower. The cervical average value was found outside the orbit and lay much higher and more ventral. The registrations taken at 80 per cent showed the average value of ventral traction to be lower and more towards the back than the one at 100 per cent RH. The cervical average value was located lower than the one at 100 per cent RH. When registered at 60 per cent RH the average ventral value was situated slightly more forward in the orbit than the value at 100 per cent RH and the cervical value was found to be a little higher than the value at 80 per cent RH.

Experimental animal C in vivo and in vitro (Figure 9)

By observing the localization of the centres of rotation in dog C, two tendencies became clear: it can be concluded that the cervical and ventral rotation centres remain relatively close to each other regardless of the degree of humidity *in vitro*. Moreover, they were located within or very near to the orbit. The same goes for the *in vivo* situation. In the *in vivo* situation, the cervical average value was found in the orbit close to the anterior border, whereas the ventral value was found lower and a little more backwards. At 100 per cent RH the cervical average value was found more towards the back of the orbit compared to the *in vivo* situation with the ventral value being located slightly higher (central). At 80 per cent RH the cervical average moved a little higher in

Table 1 Statistical differences between *in vivo* and *in vitro* measurements at different humidity levels (100, 80 and 60 per cent) for the three dogs tested (S = significant; NS = not significant).

		In vitro, 100 per cent RH	In vitro, 80 per cent RH	In vitro, 60 per cent RH
Dog A				
cerv. vivo	X	S	S	S
	Y	S	S	S
protr. vivo	X	S	S	S
	Y	S	S	NS
Dog B				
cerv. vivo	X	S	S	S
	Y	NS	S	S
protr. vivo	X	S	S	S
	Y	S	S	S
Dog C				
cerv. vivo	X	S	S	S
	Y	S	S	S
protr. vivo	X	NS	S	S
	Y	NS	S	S

the orbit. The ventral average value was found more towards the back and higher compared with the 100 per cent RH values. At 60 per cent RH the cervical average value moved further towards the back (compared with the 80 per cent RH values) and the ventral value higher and more towards the middle (compared with the 80 per cent RH values).

Comparison of the in vivo situation with the 'cranium'—measurements at different humidity levels

The statistical difference between the different 'clouds of points' was tested by means of Student's *t*-test for both coordinates. The application of various levels of humidity did not incur statistically significant changes in location of the centres of rotation when compared with the *in vivo* situation. In Table 1 the differences between the localization of the centres of rotation at different humidity levels and those found in the living animal are listed. For both coordinates of the centre of rotation a Student's *t*-test was undertaken. Only in dog C was there no difference in the location of the centres of rotation *in vivo* and in the cranium at 100 per cent RH. In all the other cases, the clustering of the centres of rotation *in vitro* and *in vivo* at least for one coordinate value, were significantly different.

Discussion

Based on the findings of these experiments, it can be concluded that:

1. The location of the centres of rotation after initial force application *in vitro* seem not to be influenced by manipulation of temperature if the temperature remains within its normal boundaries. It has to be emphasized, however, that in this pilot study, this parameter was tested for only one dog.
2. Initial displacements of the maxilla *in vitro* at different humidity levels do not coincide with those measured *in vivo*.
3. When high-pull traction was applied *in vivo* the degree of displacement of the maxilla could not be registered reliably with the method used. The same holds true for the *in vitro* measurements. Ventral as well as cervical traction cause dislocation of the sutures, resulting in more obvious initial displacements that can be registered by the method described above. On the other hand, by using high-pull forces the sutures are compressed to some extent and therefore initial displacements are difficult to measure. This is in agreement with the findings of Kragt (1979), Kragt and Duterloo (1982, 1983), and Kragt *et al.* (1982, 1984). Higher forces *in vitro* were not applied in an attempt to avoid permanent deformation of the sutures (so-called cracks).
4. No influence of the magnitude of force applied was found in the positions of the centres of rotation.
5. The sequence in which the different directions of traction were applied had no influence on the positions of the centres of rotation.
6. The tendency of the centres of rotation at lower degrees of RH *in vitro* (drying of the cranium) to move away from the cranium could be explained by the increased friction of the sutures due to a decrease in flexibility of the bone trabeculae after drying. However, it was not possible to test this hypothesis in this experiment.
7. Due to the discrepancy in the positions of the centres of rotation for the different craniums, the individual cranium morphology may have some influence. This parameter will be examined further in animals of the same litter.
8. Although the wet skull seems to react in a way more comparable to the *in vivo* situation than does the dry skull (De Clerck *at al.*, 1990), wetness does not appear to improve correlation between *in vivo* and *in vitro* behaviour adequately, as far as locating the centre of rotation is concerned.

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