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The effect of maceration on the dental arches and the transverse cranial dimensions: a study on the pig

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SUMMARY The dimensional change of the dental arches and the transverse cranial dimensions were studied in the pig to gain information on cranial post mortem changes and thus improve the possibilities of comparison between modern and skeletal samples. Dental arch dimensions were registered in 17 pigs within 30 minutes after they had been killed. The following day, the skulls were registered on lateral and axial radiographs. The animals were prepared, and storage and preparation included freezing and treatment in hot water. The water temperature did not exceed 65°C. After this process, the skulls were again registered on lateral and axial radiographs. The skulls were then allowed to dry for 2 weeks and the direct measurements were repeated. The dimensions showed shrinkage of between 0 and 3.3 per cent. The mandible showed a greater change transversally in the posterior region than the cranium, which may have been due to its shape. The more deviant values for dimensional change were probably due to technical errors and the shrinkage may be expected to vary from 0.3 to 1.7 per cent, with greater values occurring in the posterior transverse parts of the mandible. A differential shrinkage in the maxilla could not be excluded and the values varied between 0.3 and 1.9 per cent. The results indicate that the crania in skeletal samples can be expected to be 0.3–1.7 per cent smaller than *in vivo*.

Introduction

Cranial materials from different periods have been used to assess occlusal and cranial dimensions (Begg, 1954; Helm and Prydsö, 1979; Varrela, 1992; Luther, 1993; Harper, 1994). Comparison with contemporary material is of interest for the study of alterations in occlusion and arch dimensions. Some changes have been observed, such as a narrower maxilla in a modern material compared with a skeletal sample (Lysell, 1958). These comparisons rely on the assumption that there are no, or only small, skeletal changes post mortem. Dimensional changes might have occurred post mortem. The dehydration process in the bone is probably of importance. Dry skulls exposed to increased levels of humidity show some dimensional increase (Utermohle *et al.*, 1983).

Trends in prevalence and the appearance of malocclusion have been suggested (Corruccini, 1984; Corruccini and Lee, 1984; Weiland *et al.*, 1997; Brin *et al.*, 1998). Another trend in humans that has been observed and documented is the change in stature (Cernerud, 1993; Hauspie *et al.*, 1997; Hoppa and Garlie, 1998; Padez and Johnston, 1999). To evaluate such trends, comparison with older material and skeletal samples is relevant. The possible influences of post mortem changes on the arch dimensions therefore have to be evaluated.

One possible way to gain information would be to compare animals before and after a preparation process. Such a process includes removal of soft tissue. The change in humidity during this process probably affects the skeletal dimensions and mechanical properties, as is seen in the reverse process when skulls are exposed to increased levels of humidity (Albrecht, 1983; Remmelink, 1989; De Clerck *et al.*, 1990; Govaert and Dermaut, 1997). It is impossible to completely simulate the conditions at burial sites where the skeletons have been lying for a very long time. A preparation process including low and high temperatures within reasonable limits without changing the appearance of the crania is a possibility to maximize changes that can occur at burial sites. In an early work on humans (Todd, 1923), the dimensional change after maceration was investigated. That study used bisected skulls, a method that was claimed not to influence the measurements. There was no intention to describe dental arch measurements and no statistical evaluation was performed. Information on dental arch behaviour and cranial dimensions post mortem is scarce.

The purpose of the present investigation was to study the effect of the preparation process on cranial and dental arch dimensions in the pig.

Materials and methods

Seventeen pigs, *sus scrofa*, 22 months old that had been living on a small farm were used in this study. They belonged to a native breed known as the Linderoed Pig. The pigs were killed at the slaughterhouse in Skara, Sweden in a conventional procedure. Care was taken to avoid destruction of the head (Table 1). The heads were mounted on a table and direct intra-oral measurements were taken. The transverse distances between the first permanent molars were measured at the gingival margin. The measurements were taken both buccally and lingually, thus giving the maximum and minimum distances. This was also carried out for the maxillary second permanent molar, the maxillary first permanent premolar, and the mandibular first permanent premolar. The number of registrations for the premolars was less due to missing or lost premolars post mortem. The mandibular second permanent molar was registered buccally but not lingually the first time due to difficulties in gaining access. The molars were registered at the mesial root. The distance between the mesial surface of the first permanent molar and the distal surface of the permanent canine was measured in the maxilla and in the mandible on the left and right sides. The gonion area was prepared and the transverse distance between left and right gonion points was registered. Gonion was defined as the constructed point of the intersection of the ramus plane and the mandibular plane at the outer border of the mandible. All measurements were taken within 30 minutes after the pig had been killed. The measurements were made with sliding callipers to the nearest 0.1 mm.

Table 1 Processing stages and registrations.

Processing stages	Registrations
Slaughter	Direct measurements
The day after	Radiographs
Storage	Frozen
Preparation	Rinsing in cold water 2 days
	Warm water max 65°C 1.5–2.5 days
	Radiographs
Drying 2 weeks	Direct measurements

Lateral and axial radiographic cephalograms were obtained before and after the preparation procedure. The first exposures were carried out the day after slaughter, and the second exposures after the preparation processes were completed 4 months later.

The skulls were frozen until preparation was performed. The preparation began with removal of most of the superficial soft tissue. The mandible was separated from the cranium and both the mandible and cranium were kept in cold water for 2 days to rinse off the blood. The water was changed frequently and the skulls were then placed in warm water not exceeding 65°C. This water was also changed several times. The hot water procedure lasted 1.5–2.5 days, the longer time for the cranial part to allow the remaining soft tissues to be removed.

All the skulls were kept at high humidity in plastic bags until the second exposure. The lateral cephalograms were taken with a film focus distance of 1.5 m. An identification ring 27.5 mm in diameter was fixed around the right os zygomaticum and served as an enlargement control. The exposure values were 90 kV, 13 mAs for the lateral films during the first exposure with the soft tissues in place. The exposure values after the preparation process were 64 kV, 8 mAs for the lateral films. The axial films were exposed at 1.25 m, 64 kV, 8 mAs on the first occasion and 72 kV, 3.2 mAs on the second occasion. The axial films were taken with the head placed on the cassette and the second exposures were made with distance holders of 3 cm to adjust for the soft tissue. Due to the soft tissue and the long snout in the pigs, the anterior part of the snout showed different degrees of enlargement. This was a result of soft tissue that caused an upward rotation in the first films. Therefore, the anterior part of the axial films was not used. In the dorsal parts, this effect was less and the soft tissues were more evenly distributed. The identification ring was placed in the zygomatic region where the registrations on the axial films were made. The superior–inferior placement of the identification ring was registered on the lateral films to calculate enlargement deviations in the axial films. The radiographs were hand traced and direct measurements were taken to the nearest 0.5 mm. On the radiographs the distances between the right and left os zygomaticum, between the right and left mandibular sides, and the right and left maxillary permanent molars were registered. The measurement point in the os zygomaticum was chosen posterior to the sinuses visible in the os zygomaticum. The measurement point in the mandible was selected where the line connecting the distal surfaces of the lower third molars crossed the outer border of the mandible. The distance between the maxillary permanent molars was measured at the inner surfaces of the molars. These points were selected to enable identification on the pairs of radiographs (Figure 1).

The direct measurements after the maceration process were made 2 weeks after the second radiographic registration. During these 2 weeks the skulls were stored outside the plastic bags and they were thus allowed to dry. The same measurements were carried out as on the first occasion and were taken at the earlier gingival margin level. No inter-maxillary registrations were made. The measurements before and after the preparation process were compared using a paired *t*-test.

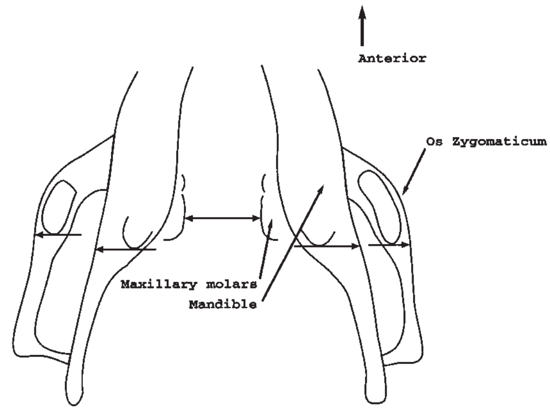

Figure 1 Measurement points used on the axial radiographs. The measurement point on the os zygomaticum was chosen posterior to the sinuses visible in the os zygomaticum. The measurement point on the mandible was selected where the line connecting the distal surfaces of the lower third molars crossed the outer border of the mandible. The distance between the maxillary permanent molars was measured at the inner surfaces of the molars. These points were selected to enable identification on the pairs of radiographs.

The measurements on the skulls were repeated after 2 weeks in order to assess the error of the measurement. The tracings were again measured and compared with the original measurements. Sixteen of the radiographs were retraced and re-measured in order to evaluate the error of landmark identification. The error of the measurement was calculated using the formula $S_e = \sqrt{S_d^2/2}$ (Houston, 1983). Systematic errors were calculated using a paired *t*-test. The level of significance was set at 5 per cent.

Errors of the measurements

The errors of the measurements are listed in Table 2. There was a systematic error when the two tracings were compared for the mandibular and the intermolar measurement. The first measurements were 0.3 mm larger compared with the second series. The other measurements showed no systematic errors.

Results

The maxillary registrations before and after preparation and drying are shown in Table 3.

Table 2 Errors of the measurements.

Direct measurements	mm
Maxillary second permanent molar buccally	0.3
Maxillary second permanent molar lingually	0.2
Maxillary first permanent molar buccally	0.3
Maxillary first permanent molar lingually	0.2
Maxillary first permanent premolar buccally	0.3
Maxillary first permanent premolar lingually	0.3
Distance maxillary first permanent molar to canine on the right side	0.4
Distance maxillary first permanent molar to canine on the left side	0.4
Mandibular second permanent molar buccally	0.5
Mandibular first permanent molar buccally	0.4
Mandibular first permanent molar lingually	0.4
Mandibular first permanent premolar buccally	0.4
Mandibular first permanent premolar lingually	0.3
Distance mandibular first permanent molar to canine on the right side	0.4
Distance mandibular first permanent molar to canine on the left side	0.3
Bigonial	0.5
Radiographic measurements, same tracing	
Transversal distance of the zygomatic bone	0.3
Transversal distance of the mandible	0.2
Maxillary intermolar distance	0.2
Radiographic measurements, duplicate tracing	
Transversal distance of the zygomatic bone	0.5
Transversal distance of the mandible	0.3
Maxillary intermolar distance	0.3

The mean values showed a shrinkage of 0–1.3 mm, those below 0.4 mm not being statistically significant. The largest differences were seen in the transverse molar measurements registered lingually. The relative change was larger in the lingual than in the buccal registrations.

The mandibular values before and after the preparation process are shown in Table 4. The mean values for the dental arch measurements showed a shrinkage of 0.6–0.9 mm. The measurement between the left and right gonion points showed a larger shrinkage, 3.9 mm. The relative change was also larger in the bigonial registration.

The values before and after the preparation process evaluated on the radiographs are shown in Table 5. There was a mean shrinkage of 0.7–2.1 mm.

The mean distances of the buccal and lingual transverse measurements for the three maxillary and two mandibular measurements are shown in Table 6. The mean shrinkage was 0.4–0.8 mm, with both maxillary and mandibular values showing the same trend.

Discussion

The dental arch and transverse skeletal dimensions before and after maceration in the pig were studied. The evaluation was performed using direct measurements and indirect radiographic assessment. Shrinkage occurred in all registered dimensions except for the maxilla, where the distance from the first permanent molar to the permanent canine showed an insignificant change in the same direction. There was also no difference in the transverse distances between the buccal surfaces of the maxillary first permanent molar or between the buccal surfaces of the maxillary first permanent premolars, but this could be due to technical errors. The other parameters, apart from those related to the maxillary teeth transversally, showed a shrinkage of 0.3–2.7 per cent. The transverse mandibular skeletal value showed greater relative changes in the gonion area and accounts for the 2.7 per cent change. The other parameters decreased by 0.3–1.9 per cent.

Table 3 The maxillary distances before and after the maceration process and the difference between these values.

	n	Before		After		Difference		*P*	95% confidence interval for the mean difference	Change %
		Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD			
Second permanent molar buccally	17	72.0	2.23	71.6	1.99	0.4	0.47	**	0.1–0.6	0.5
Second permanent molar lingually	17	39.7	1.64	38.4	1.84	1.3	0.74	***	0.9–1.7	3.3
First permanent molar buccally	17	69.6	2.06	69.6	2.16	0.0	0.84		–0.4–0.5	0.0
First permanent molar lingually	17	42.3	1.89	41.1	1.79	1.3	0.72	***	0.9–1.7	3.0
First permanent premolar buccally	15	62.2	2.65	61.9	2.85	0.3	0.51		0.0–0.5	0.4
First permanent premolar lingually	15	54.4	2.43	53.6	2.43	0.6	0.86	*	0.2–1.2	1.2
Distance first permanent molar to canine on the right side	17	63.4	3.13	63.1	3.27	0.3	0.92		–0.2–0.8	0.5
Distance first permanent molar to canine on the left side	17	62.9	2.79	62.7	2.97	0.2	0.75		–0.2–0.5	0.3

The transverse distance was measured at the gingival margin from the buccal surface and at the gingival margin from the lingual surface.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 4 The mandibular distances before and after the maceration process and the difference between these values.

	n	Before		After		Difference		*P*	95% confidence interval for the mean difference	Change %
		Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD			
Second permanent molar buccally	17	70.2	1.81	69.5	1.93	0.7	0.87	**	0.3–1.2	1.0
First permanent molar buccally	17	68.9	2.14	68.1	1.86	0.7	1.11	*	0.1–1.3	1.0
First permanent molar lingually	17	46.6	1.62	45.9	1.52	0.7	1.08	*	0.1–1.2	1.5
First permanent premolar buccally	14	56.0	3.89	55.4	3.90	0.6	0.64	**	0.2–0.9	1.0
First permanent premolar lingually	14	48.6	3.47	47.9	3.71	0.7	0.64	**	0.3–1.1	1.5
Distance first permanent molar to canine on the right side	17	67.5	3.27	66.6	3.31	0.9	0.74	***	0.5–1.3	1.3
Distance first permanent molar to canine on the left side	17	67.5	3.39	66.5	3.34	0.9	0.89	***	0.5–1.4	1.4
Bigonial	17	144.1	5.91	140.2	6.57	3.9	1.73	***	3.0–4.8	2.7

The transverse distance was measured at the gingival margin from the buccal surface and at the gingival margin from the lingual surface.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 5 The transverse distance before and after the maceration process registered on radiographs.

	n	Before		After		Difference		P	95% confidence interval for the mean difference	Change %
		Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD			
Transversal distance of the zygomatic bone	17	171.2	7.2	169.9	6.6	1.3	1.21	***	0.7–2.0	0.8
Transversal distance of the mandible	17	125.2	5.1	123.1	4.7	2.1	1.48	***	1.3–2.8	1.7
Maxillary intermolar distance	17	34.3	1.7	33.7	1.5	0.7	0.42	***	0.4–0.9	1.9

***$P < 0.001$.

Table 6 The mean values of the transverse differences between buccal and lingual measurements. Difference between before and after the preparation process.

	n	Before		After		Difference		P	95% confidence interval for the mean difference	Change %
		Mean (mm)	SD	Mean (mm)	SD	Mean (mm)	SD			
Maxillary second permanent molar	17	55.8	1.79	55.0	1.83	0.8	0.40	***	0.6–1.0	1.5
Maxillary first permanent molar	17	56.0	1.93	55.3	1.92	0.7	0.61	***	0.3–1.0	1.2
Maxillary first permanent premolar	15	58.2	2.42	57.8	2.57	0.4	0.52	**	0.2–0.7	0.8
Mandibular first permanent molar	17	57.7	1.80	57.1	1.59	0.7	0.99	*	0.2–1.2	1.2
Mandibular first permanent premolar	14	52.3	3.68	51.7	3.80	0.6	0.58	**	0.3–1.0	1.2

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

The relatively lesser shrinkage in the mandible assessed on the radiographs compared with the direct measurements is partly due to the different measurement points; those on the radiographs were made anterior to gonion. Due to the shape of the mandibular body, it is possible that there is a greater relative change in the posterior parts (Albrecht, 1983). A factor contributing to the difference between the radiographic and direct measurements was the different levels of humidity. The skulls were kept in plastic bags in high humidity until the second radiographs were taken, but as some skulls had become mouldy they were subsequently kept in a dry condition. The measurements on the skulls were performed after about 2 weeks, and this is the most probable explanation for the differences in the mandibular registrations. The drying process caused further shrinkage of the skulls from the day of radiographic registration to the day of the direct measurement. Gonion is unambiguous in the transverse direction in the pig. It should also be noted that the direct measurements made on the mandibular molar teeth and the radiographic transverse mandibular registration posterior to this region do not differ to any great extent. The bigonial measurement taken directly, which showed a larger change, was registered in a part where the bone is thinner and this probably contributes to the change seen in this region.

In a study of the effect of humidity performed on 10 *Macaque* skulls, the molar dentition was found to increase in length by 0.50 per cent, and the greatest length of the skull increased by 0.57 per cent when the skulls had been exposed to increased humidity for 24 hours (Albrecht, 1983). The width at the condyles did not show the same uniform behaviour. One possible explanation proposed was that this measurement was the only one that did not span bone. Todd (1923) measured 24 freshly macerated bisected human skulls 12 hours after they emerged from the macerating tank, probably at maximum humidity, and he repeated the measurements at 1-week intervals thereafter. He found an average shrinkage in length of 0.83 per cent, in breadth of 1.23 per cent, and in height of 1.32 per cent when the before and after measurements were compared. The changes were greatest in the first weeks and only minor changes were seen after 4 weeks. The change with these figures combined gives a shrinkage of 1.0 per cent in length, 1.5 per cent in breadth, and 1.5 per cent in height. It was noted that no warping occurred during drying in these series.

Measurements of changes in size have been made on two human skulls at different levels of humidity; the maximum relative humidity level in the experiment was 98 per cent (Utermohle *et al.*, 1983). Some variables were invariant and the mean length of these measurements was 34 mm. The humidity sensitive measurements averaged 99 mm. One of the skulls was treated with a coating, and the different levels of humidity influenced this specimen less. The increase in relative humidity in this experiment was 80 per cent up to the maximum level of 98 per cent relative humidity. The average increase of the affected variables in the untreated specimen was 1.5 mm, whereas the treated specimen had an average increase of 1.1 mm. The values decreased when the level of humidity was lowered to normal values. These extreme variations, registered in two skulls only, indicate an expansion of 1.5 per cent at the highest level of humidity compared with the values registered at low levels (less than 18 per cent) of relative humidity.

In the present investigation the direct measurements differed in the maxillary intermolar distances between the external and internal distances. Both maxillary first and second intermolar distances measured lingually showed greater differences than the corresponding buccal measurements. This could either be a true difference or a technical error. If a technical error is assumed, it could be due to the difficulty of correct landmark identification. The measurements during the slaughter process were made at the gingival margin and, although this point was relocated in the prepared skull, there is a certain risk that the second series of measurements was performed in a more apical direction. This would explain the difference (Figure 2). To overcome some of these difficulties, the mean of the external and internal measurements is presented in Table 6. These values probably represent the true shrinkage of the maxilla.

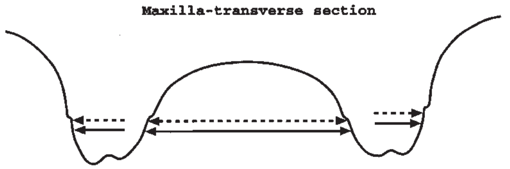

Figure 2 The technical measurement error in this study. The second measurement has been performed in a more apical direction than the first measurement due to difficulties in gaining access in the first series. If an equal amount of shrinkage of the buccal and the lingual points is assumed, then this explains why there is no change in the external values but relatively larger values in the internal values. This is proposed as the most probable explanation.

Another possibility also representing a true difference would be palatal shrinkage in the superior part of the maxilla, which would contract the apical more than the coronal parts of the teeth and thus change the torque to some extent. This change in torque would also tend to change the measurement points in an apical direction lingually. A contraction such as this would then be the reverse of that seen in rapid maxillary expansion, which is not likely (Braun *et al.*, 2000). The results show that there is a decrease in the transverse dental arch dimensions of about 0.8–1.5 per cent in the direct measurements if a technical error is assumed. Compared with the radiographic evaluation, where the measurements were made at the lingual side, the mean difference of the buccal and lingual registrations is in accordance with the radiographic evaluation. On the other hand, it is likely that there has been further shrinkage between the radiographic registrations and the direct measurements due to the earlier mentioned drying process. This means that 'differential' shrinkage, giving a change in torque of the molars, cannot be ruled out. It must be remembered that the measurement points are not the same in the direct and radiographic measurements. The radiographic evaluation was performed at the third molars, which were best identifiable in pairs. A larger apical movement could be assumed for the third molars since they were not completely developed and an unerupted tooth has a larger area of soft tissue around it. The method error is also probably larger in the radiographic measurements.

The canine, used in the lateral arch measurements, is often movable in the axial direction. The measurement on the dry skull was made with this tooth in its most apical position. This is the only possible approach and the error due to this is probably of less magnitude than that due to identification of the gingival margin.

The shrinkage is not invalidated by the errors of the measurements. Some of the mean values show a smaller change in the range of the errors, but these are also not statistically significant. The three measurements where the 95 per cent confidence intervals include the possibility of an expansion are the transverse maxillary distance between the first permanent molars registered buccally, and the distances between the maxillary first permanent molar and the canine on the right and left sides. The transverse distance is better described by the mean values of external and internal measurements, and taking only the buccal registration into account probably includes a technical error. The distances between the maxillary first permanent molar and the canine on the right and left sides are different and show no significant change. However, the present investigation does not indicate expansion in any of the measurements made. Some of the variation in the results is explained by the size of the errors of the measurements in relation to the size of the differences.

Radiographic evaluation presents problems of enlargement and distortion (Ahlqvist *et al.*, 1986, 1988). The measurements used in the present study were taken in proximity to the enlargement calibration ring. This ring was also large, to facilitate calculations of the enlargement. The enlargement was adjusted for between the two registrations using the enlargement of the ring and the placement of the ring on the lateral radiograph. Landmark identification is also a problem in cephalometry (Midtgård *et al.*, 1974; Houston *et al.*, 1986). Care was taken to select landmarks that were identifiable on both cephalograms. The size of the error of the measurements on the radiographs is in accordance with the least error found for the length measurements in previous studies (Midtgård *et al.*, 1974; Sandler, 1988). This was due to the fact that easily identifiable landmarks were selected for the present work. Similar methods of calculating the enlargement from cephalograms taken from different views have been used previously (Hsiao *et al.*, 1997; Iseri and Solow, 2000).

Direct comparison with skeletal samples of human origin is not possible since the human samples had been left for long periods of time. There is a great variation in this respect as some studies concern skeletons from more recent times (Ingervall *et al.*, 1972; Weiland *et al.*, 1997), and other studies, older samples (Lunt, 1969; Varrela, 1990; Luther, 1993; Harper, 1994). However, the treatment of the pig skulls in this experiment was quite harsh and included higher temperatures than those occurring in nature. Lower temperatures are probably less important. Freezing did not affect the stiffness of trabecular bone in a study of different storage methods and their influence on the mechanical properties (Linde and Sorensen, 1993). The dehydration is probably the main cause of the observed dimensional changes. The findings in this investigation in pigs show a uniform picture of a slight decrease in dental arch and cranial dimensions after maceration. These findings are in agreement with those of Todd (1923) on external cranial dimensions in humans. They do not make earlier reports on skeletal samples invalid. Expansion was not seen in this study.

Conclusions

Transversal dimensional changes after maceration were studied in the pig. The dimensions showed shrinkage of between 0 and 3.3 per cent. The outliers were considered to be technical errors and the normal mean shrinkage was found to be in the range 0.3–1.9 per cent. The mandibular gonion area showed a greater change transversally, 2.7 per cent. This is attributed to the mandibular body shape and the size of the supporting bone in this region. The value of 1.9 per cent could include a soft tissue component and a larger technical error in the non-erupted teeth registered radiographically. The mean shrinkage in the other registrations was 0.3–1.7 per cent.

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