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Contents

The Identification of Bite Marks Using the Reflex Microscope

A. J. Ligthelm, W. J. C. Coetzee and P. J. van Niekerk

Faculty of Dentistry, University of Pretoria. Pretoria 0001, South Africa

Abstract

Bite marks in, for example, cheese, butter and chewing-gum are often difficult and sometimes impossible to identify due to shortcomings in present techniques. In this study the reflex microscope was used to evaluate and identify bite marks in apples, cheese and chewing-gum. Positive identification using the reflex microscope has become possible with a high level of accuracy. Three impression materials were also evaluated for the damage they cause to bite marks and it was found that Reprosil (LD Caulk Co, Milford, Delaware 19963, USA) and President (Coltène AG, CH-9450 Altstätten, Switzerland) did not cause any damage, but an auto-polimerising Bis-GMA resin, Concise (Dental Products/3M, St. Paul, MN 55144, USA) did damage the bite marks.

Mathematical, computerised three-dimensional comparisons between bite marks and the dentition of suspects using the reflex microscope proved to be possible and very successful. Multiple measurements could be carried out and stored electronically for later use. The technique was simple and it was possible to retain the bite mark in its undamaged state.

Introduction

The unique morphological and alignment characteristics of the human dentition and bite marks are well documented.[1–5] It sometimes occurs that bite marks are found in cheese, butter, chewing-gum or apples at the scene of a crime, and which are often difficult if not impossible to identify using present methods.[6–7] In spite of the controversy regarding the reliability of bite mark analysis, the validity of specific procedures and criteria used for the identification of bite marks has gained acceptance in the courts.[7]

Although acceptance of bite mark evidence has progressed, there is still a constant search for new methods which may improve on the shortcomings of traditional techniques. Recent methods described include infra-red and ultra-violet light photography, scanning electron microscopy, computerized image enhancement, radiographic techniques, stereometric graphic plotting and the use of three-dimensional measuring instruments.[8–12] Shortcomings of these methods include inaccurate visual, photographic or graphic matching and damage to the bite mark due to certain procedures such as the making of impressions.

The purpose of this study was to evaluate the possible application of the reflex microscope[13] in order to identify the dentition responsible for bite marks made in cheese, chewing-gum and apples.

Materials and Methods

1. The reflex microscope

The principle of the reflex microscope is illustrated in Fig. 1. The object on the right is reflected in a semi-silvered mirror. An object point P creates an image of itself at R, where PR is normal to the mirror and P and R are equi-distant from it. The reflected image is thus seen to contain all the three-dimensional properties of the original object. An observer looking at R is able to see a measuring mark (light dot) M through the mirror and when M coincides with R visual parallax is eliminated. The observer can thus move M about the image, taking x, y, z co-ordinates as required, and the object, which is stationary, does not obstruct the movement of M. This principle was used in developing the reflex microscope (Fig. 2). The x, y, z co-ordinates of points observed with the reflex microscope are converted into electronic signals and recorded on an IBM XT computer with programmes specifically designed to accept them. These point co-ordinates of object profiles can then be evaluated by using computerised mathematical models for comparing the numerous coinciding characteristics of a bite mark and the dentition that could have caused it.

To be able to match the bite mark and the cast of the dentition, the x, y, z co-ordinates of the bite mark profile were transformed mathematic-

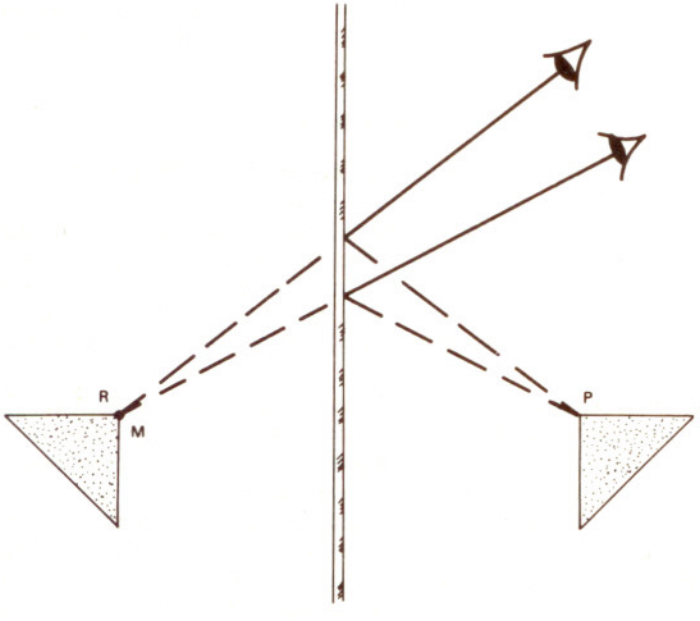

Fig. 1. The reflex principle.

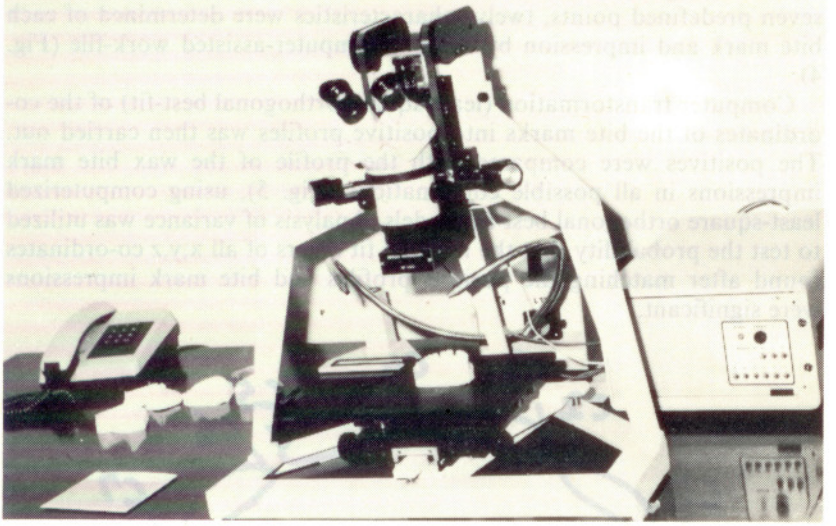

Fig. 2. The Reflex Microscope.

ally with the computer programmes to produce a "positive" contour profile of the bite mark. This positive was visually compared with the profile of the dentition cast.

2. **Contour mapping of co-ordinates**

The first part of the study consisted of the reflex microscopic evaluation of a bite mark and the dentition suspected of having caused it. Occlusal and incisal surfaces were predefined on the bite mark and cast of the dentition before evaluation. After approximately 600 x, y, z co-ordinates were determined on both cast and bite mark, contour mapping of these co-ordinates was carried out with the aid of the computer.

3. **The effect of impression materials on the bite marks**

In the second part of the study bite marks were made in dental wax, cheese, chewing-gum and apples by a subject. The bite marks and their impressions were evaluated with the reflex microscope before and after impressions were taken with President*, Reprosil** and an auto-polimerising Bis-GMA resin, Concise***.

Evaluation of the bite marks and impressions in the second part of the study differed from the procedures in the first part of the study in that only seven predefined points on the bite marks and impressions were evaluated with the reflex microscope instead of 600 points. The seven points on the bite marks and therefore also on the impressions were selected according to the unique morphological and alignment

* Coltène Ag, CH-9450 Altstätten, Switzerland.
** LD Caulk Co., Milford, Delaware 19963, USA.
*** Dental Products/3M, St. Paul, MN 55144, USA.

characteristics of the incisal and occlusal surfaces of the dentition involved (Fig. 3). After evaluation with the reflex microscope of the seven predefined points, twelve characteristics were determined of each bite mark and impression by using a computer-assisted work-file (Fig. 4).

Computer transformation (least-square orthogonal best-fit) of the co-ordinates of the bite marks into positive profiles was then carried out. The positives were compared with the profile of the wax bite mark impressions in all possible combinations (Fig. 5), using computerized least-square orthogonal best-fit models. Analysis of variance was utilized to test the probability that the residual fit errors of all x,y,z co-ordinates found after matching the positive profiles and bite mark impressions were significant.

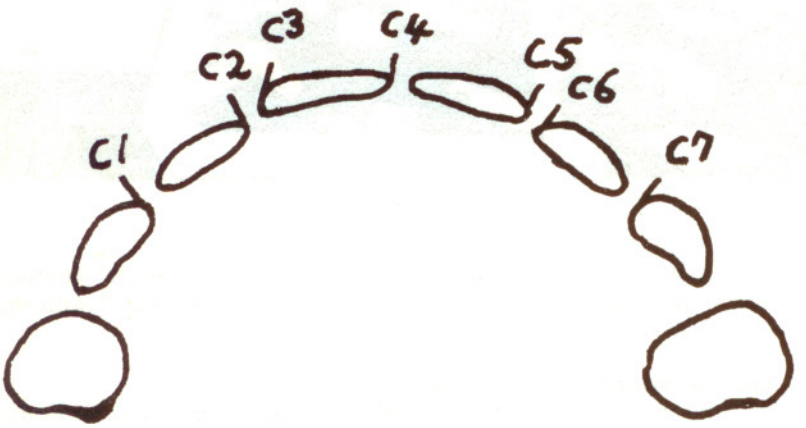

Fig. 3. Diagram of selection of points evaluated.

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1.  Observe points C1  C2  C3  C4  C 5  C6  C7
2.  Distance between C1 - C2
3.  Distance between C2 - C3
4.  Distance between C3 - C4
5.  Distance between C4 - C5
6.  Distance between C5 - C6
7.  Distance between C6 - C7
8.  Fit line C123 to points C1  C2  C3
9.  Fit line C45 to points C4   C5
10. Distance between C1 - C7
11. Fit line C67 to points C6  C7
12. Transform observed co-ordinates
```

Fig. 4. Computer work-file and characteristics determined for each bite mark and impression.

Results

The results of the first part of the study revealed a remarkable similarity when the contour maps of the bite mark and cast were compared (Fig. 6). The vast number of measurements involved (± 600), however, add

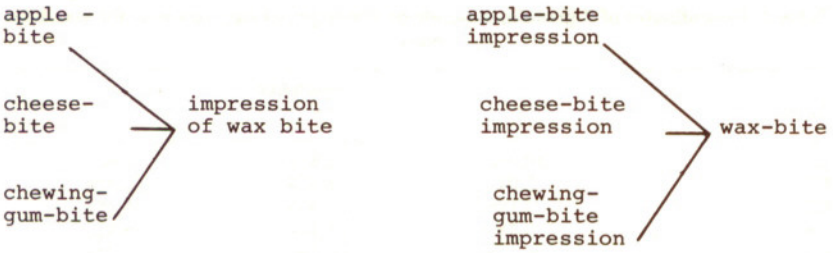

Fig. 5. Combinations of matching bite marks and impressions.

Fig. 6. Contour mapping of bite mark "positive" (upper diagram) and cast of suspected dentition (lower diagram).

up to a time-consuming task when compared with the simplicity of the second method.

Tables 1, 2 and 3 show x,y,z co-ordinates for the apple bite mark itself, and Reprosil impressions of the bite mark and wax bite mark. Table 4 shows the residual fit errors after transformation of all co-ordinates in Table 2 to the co-ordinate system in Table 1. Table 5 shows the distances between points C1-C7 on the apple bite mark, apple bite mark impression and wax bite mark impression.

Data of all the other combinations (omitted), were similar to data in Tables 1-5 which are representative of the second part of the experiment. It was observed, however, that Bis GMA impressions partially distorted and damaged the bite marks in the cheese, apple and chewing-gum. This part of the study was therefore carried our last.

Table 1. Co-ordinates of 7 predefined points on the Reprosil impression of the wax bite mark

	Co-ordinates		
Points	*x*	*y*	*z*
C1	–10,635	22,889	–6,461
C2	– 7,041	24,459	–6,316
C3	– 3,186	27,005	–6,357
C4	5,735	26,413	–5,816
C5	9,474	23,590	–5,534
C6	12,625	22,276	–5,440
C7	14,244	19,038	–5,549

Table 2. Co-ordinates of 7 predefined points on the apple-bite mark after transformation to a positive profile

	Co-ordinates		
Points	*x*	*y*	*z*
C1	–10,830	22,642	–6,491
C2	– 7,476	23,817	–6,373
C3	– 2,744	27,879	–6,192
C4	5,407	27,199	–5,928
C5	9,598	23,501	–5,572
C6	12,639	22,087	–5,401
C7	14,618	18,542	–5,439

Table 3. Co-ordinates of 7 predefined points on the apple impression

	Co-ordinates		
Points	*x*	*y*	*z*
C1	–10,016	23,960	–6,970
C2	– 6,875	24,809	–6,377
C3	– 2,668	27,108	–6,418
C4	5,148	26,411	–5,648
C5	9,092	23,195	–5,447
C6	12,436	21,781	–5,275
C7	14,091	18,397	–5,347

Table 4. Residual fit errors after transformation of all co-ordinates in Table 2 to the co-ordinate system in Table 1

	Residual Fit Errors		
Points	*x*	*y*	*z*
C1	–0,195	–0,247	–0,030
C2	–0,435	–0,642	–0,057
C3	0,442	0,874	0,165
C4	–0,328	0,786	–0,112
C5	0,124	–0,089	–0,038
C6	0,014	–0,189	–0,041
C7	0,374	–0,496	0,110

Table 5. Distances between points selected on the bite marks and impressions, as determined from the co-ordinate systems of the computer work-file

	Distance (mm)		
Points	*Apple (bite mark impression) (Reprosil)*	*Apple (bite mark)*	*Wax (impression) (Reprosil)*
C1-C2	3,307	3,555	3,925
C2-C3	4,795	6,239	4,620
C3-C4	7,885	8,183	8,957
C4-C5	5,092	5,600	4,694
C5-C6	3,635	3,355	3,324
C6-C7	3,768	4,061	3,622

$P < 0,05$

Orthognathic regression fitting of co-ordinates and distances between the previously selected points showed no significant differences ($p < 0,05$) in all three impression materials between the bite marks before and after impressions were taken, the impressions, and the "suspected dentition" cast.

Discussion

In the light of currently used methods to record and observe bite marks it is often impossible to meet all the requirements, retain the evidence and achieve identification.[6,7]

One of the most important features of the reflex microscope is that non-contact measurements in three dimensions can be made directly of bite marks otherwise not suitable for taking measurements or impressions, such as butter. In certain circumstances stereo-photographs of bite marks on a human body can be evaluated with the reflex microscope. Compared with other methods, the initial cost of the reflex microscope is high. Operating costs of the reflex microscope are, however, low and the microscope has proved to be accurate and relatively simple to operate. Computer link-up extends the versatility of this apparatus beyond any of the previously used methods.

The procedure for detailed evaluation of the bite mark as used in the first part of the experiment proved to be very successful regarding identification (Fig. 6). It is, however, time-consuming but may be the only way to identify certain bite marks. The procedure in the second part of the study proved to be very accurate, simple and quicker.

Once the predefined points on a bite mark and cast of suspected dentition have been measured with the reflex microscope, many characteristics, e.g. distance, angle, etc., can be calculated with the aid of the computer and mathematical models. This procedure on the reflex microscope is similar to present techniques where a set of characteristics of a bite mark and dentition are compared.

Conclusion

In this study the principle of three-dimensional morphological and

alignment characteristics of dentition and bite mark was successfully applied using the reflex microscope to evaluate bite marks for identification.

Mathematical, computerised three-dimensional comparisons between bite marks and the dentition of suspects proved to be possible and very successful. Multiple measurements could be carried out and stored electronically for later use. The technique was simple and it was possible to retain the bite mark in its undamaged state.

Acknowledgements

The authors would like to thank Prof. L. P. Adams of the Department of Surveying at the University of Cape Town and his personnel for their valuable assistance during the first stage of the experiment when we were waiting for the installation of our reflex microscope. We would also like to thank Dr. C. van der Merwe of the Biostatistics Unit of the SA Medical Research Council for help in interpreting the results, Mr. J. Nell for taking the photographs, and Mrs. S. W. Jacobs for typing the manuscript.

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The Frontal Sinus: Forensic Fingerprint? — A Pilot Study

A. M. P. Harris, R. E. Wood, C. J. Nortjé and C. J. Thomas

Faculty of Dentistry, University of Stellenbosch, Private Bag X1, Tygerberg 7505, Republic of South Africa

Abstract

The image of the frontal sinus, as seen on rigidly standardized occipito-mental radiographs, was analysed in 32 randomly selected patients from the same racial group. Radiographic tracings were compared and definite differences in sinus height, width, and number of edge loculations were seen between patients, with no two sinuses being alike in appearance. It is postulated that the image of the frontal sinus may be suitable as a means of identification. A method for orientation of the skull in post-mortem radiograph production is suggested.

Key words: Frontal sinus, forensics.

Introduction

The frontal sinus is a paired, irregularly shaped, pneumatized cavity located in the frontal bone deep to the superciliary arch. Each sinus communicates with the nasal fossa on the same side via the infundibulum and is separated from its partner by a boney septum.[1] Like all paranasal sinuses they originate as outpouchings of the nasal mucosa,[2] are present at birth and begin to grow slowly at five years-of-age.[3] Growth is more rapid after the onset of puberty and continues until the sinuses reach maximum size at the age of 20.[2]

Turner and Porter[4] were the first to study the anatomy of the frontal sinuses using radiographic methods. Brachycephalic crania exhibited better developed frontal sinuses than did mesocephalic crania which in turn showed more developed frontal sinuses than dolichocephalic crania. They found that the frontal sinuses were better developed in patients of the mixed European races than in those of the so-called pure races. They also found no relation between degree of development of the frontal and mastoid sinuses in any racial group. Buckland-Wright (1970) concluded that environmental or genetic factors control frontal sinus configuration within each population, but the overall size and shape of the sinus remains generally constant within a population.[5] Changes due to age, disease, gender and race[6] have also been observed.

Culbert and Law[7] stated that anthropometric radiography is useful as a record of general and criminal identification. Schuller[2] stated that the radiologic appearance of frontal, sphenoidal and mastoid sinuses show individual variation of size and configuration. Maxillary sinuses and ethmoids are not as useful for forensic purposes,[7] but when the frontal sinuses are affected by pathology the sphenoid sinus and mastoid air cells may be utilized.

The frontal sinus is visible on several extra-oral radiographic views commonly used in maxillofacial radiology. Occipitomental, postero-anterior mandible and skull views are frequently performed in this institution and are routinely taken in cases of maxillofacial trauma. The purpose of this pilot study was to determine the extent to which the radiographic image of the frontal sinus differs on individual occipito-mental radiographs.

Materials and Methods

Thirty-two patients with clinical problems requiring occipitomental radiographs were obtained from the general clinic population of the Faculty of Dentistry, University of Stellenbosch. None had presented for problems related to the frontal sinus. All radiographs were performed by the same radiography technician using the same angulation, radiographic film, cassette and processing methods. Patients were past the age of 20 years and no patient was irradiated solely for the purpose of this study. The patients were all of mixed African-Malay-European ancestry and no attempt was made to separate males and females.

The radiographs obtained were examined by two radiologists and tracings were made of each frontal sinus. These tracings were then subjectively sorted on the basis of size of the frontal sinus. The maximum vertical and horizontal dimensions were measured for each sinus, and the number of edge loculations on the superior border were counted and verified by a second observer. The superior border was used because it was most easily visible and contained the largest loculations.

Results

No two frontal sinuses were alike (Fig. 1). They differed with respect to width, height and number of edge loculations. The largest sinus was approximately 6 times the size of the smallest sinus. The median vertical dimension was 39 mm, the median horizontal width was 67 mm, and the median number of edge loculations was 5. The maximum height, width, and number of edge loculations are expressed in Table 1.

Discussion

The image of the frontal sinus was unique for each patient. In a case of identification, comparison of ante- and post-mortem radiographic images of the frontal sinus could be useful. It is not necessary to have standardized ante-mortem views because post-mortem radiographs may be matched with respect to density and angulation although several views may be necessary to obtain a correct match. The proposed method of matching ante-mortem and post-mortem sinus radiographs is described in Fig. 2.

Frontal sinus radiographs may be used in two ways for identification purposes. The choice of approach depends on whether the identity of the corpse is suspected as a result of, for example, circumstances of the

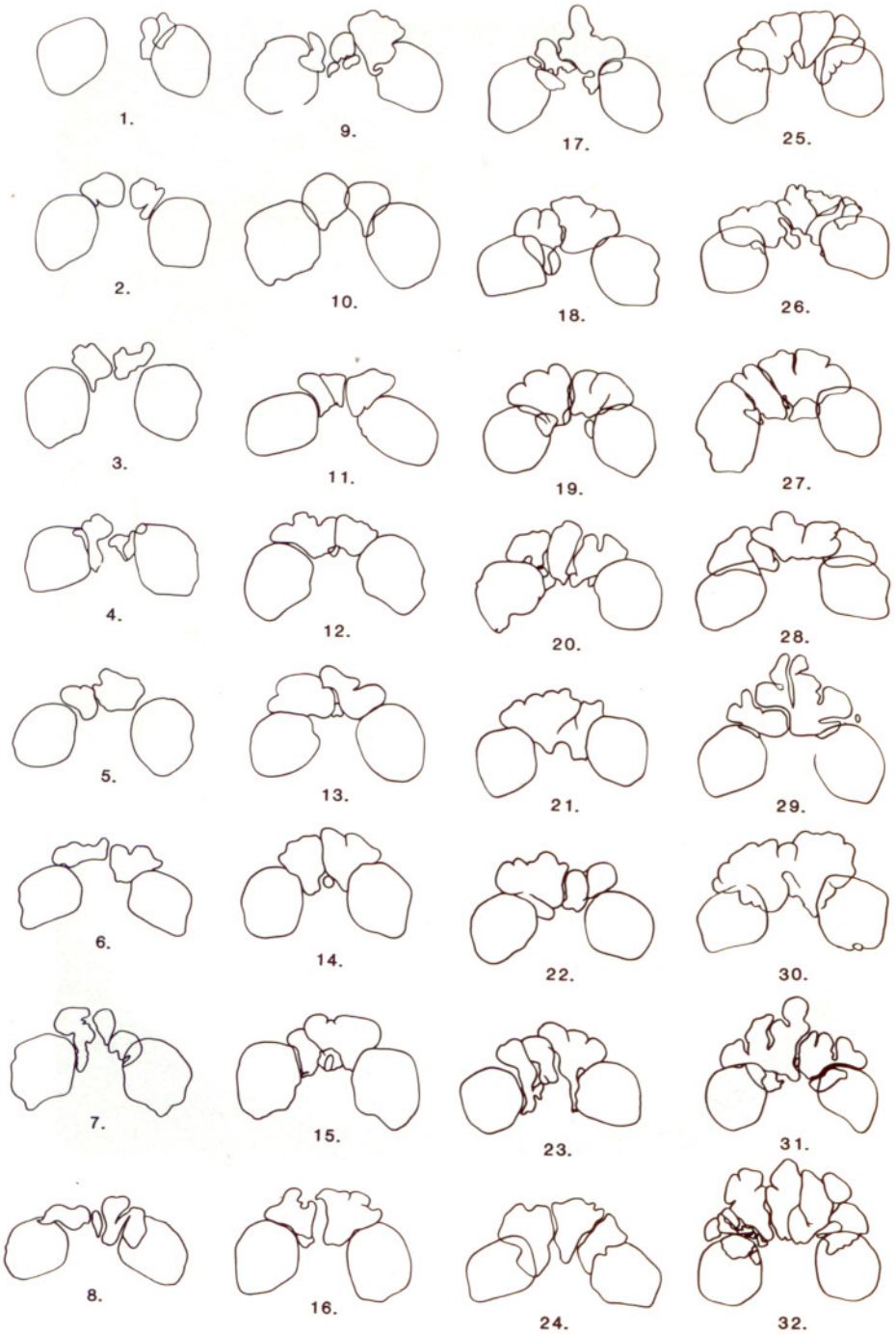

Fig. 1. Tracings of the occipitomental radiographic image of the frontal sinuses and orbits in 32 patients showing individual variation in outline and size.

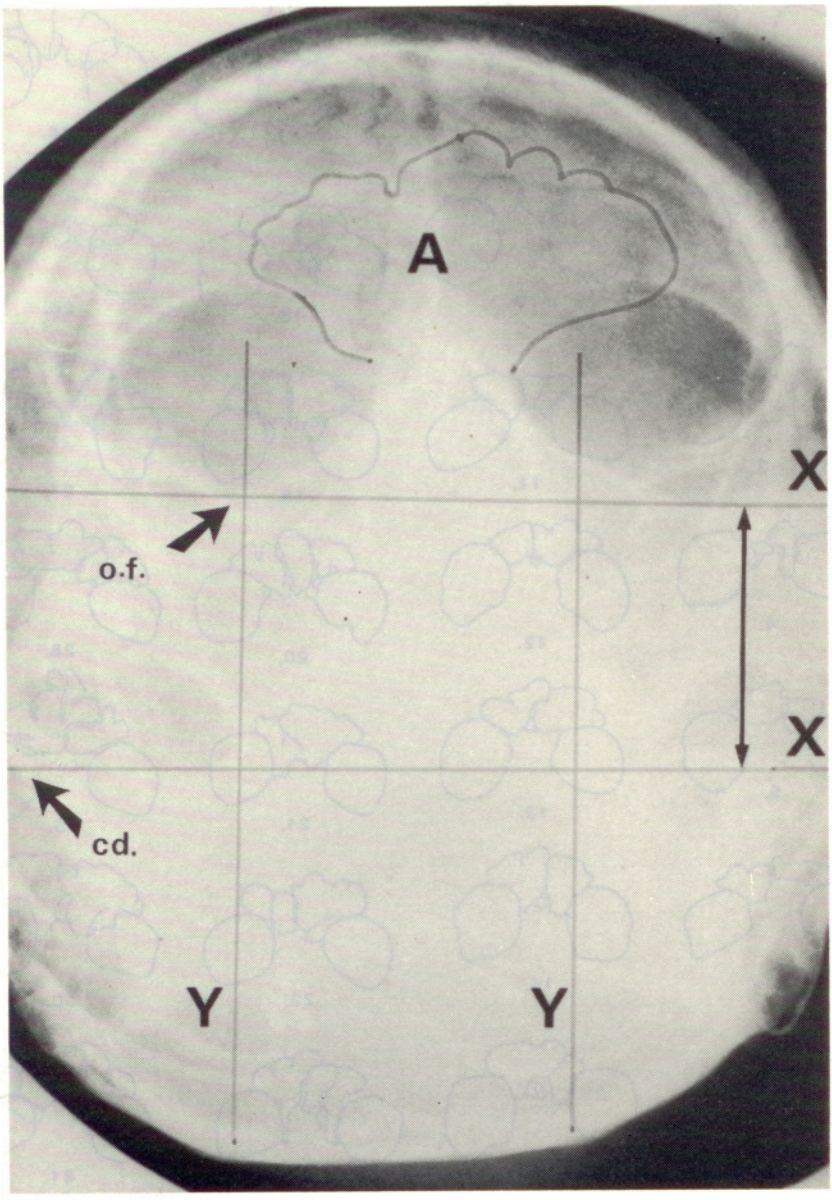

Fig. 2(A)

Fig. 2. Horizontal (x) and vertical (y) lines for ensuring similar orientation of ante- and postmortem skull radiographs. Angulation used in the production of radiograph (A) was matched in the production of radiograph (B) by ensuring equal distances between horizontal and vertical registration lines. Radiograph C shows the different radiographic appearance of the frontal sinus of the same skull at a different angulation (o.f. = optic foramen, cd. = condylar head).

death, personal effects found at the scene, or not. The required approach involves obtaining suitable ante-mortem radiographs from appropriate sources, possibly identifiable in the medical records of the deceased. If radiographs of the frontal sinus are obtained then the same views of the deceased may be taken at similar angulation and density. The ante- and post-mortem frontal sinus outlines may then be traced on orthodontic

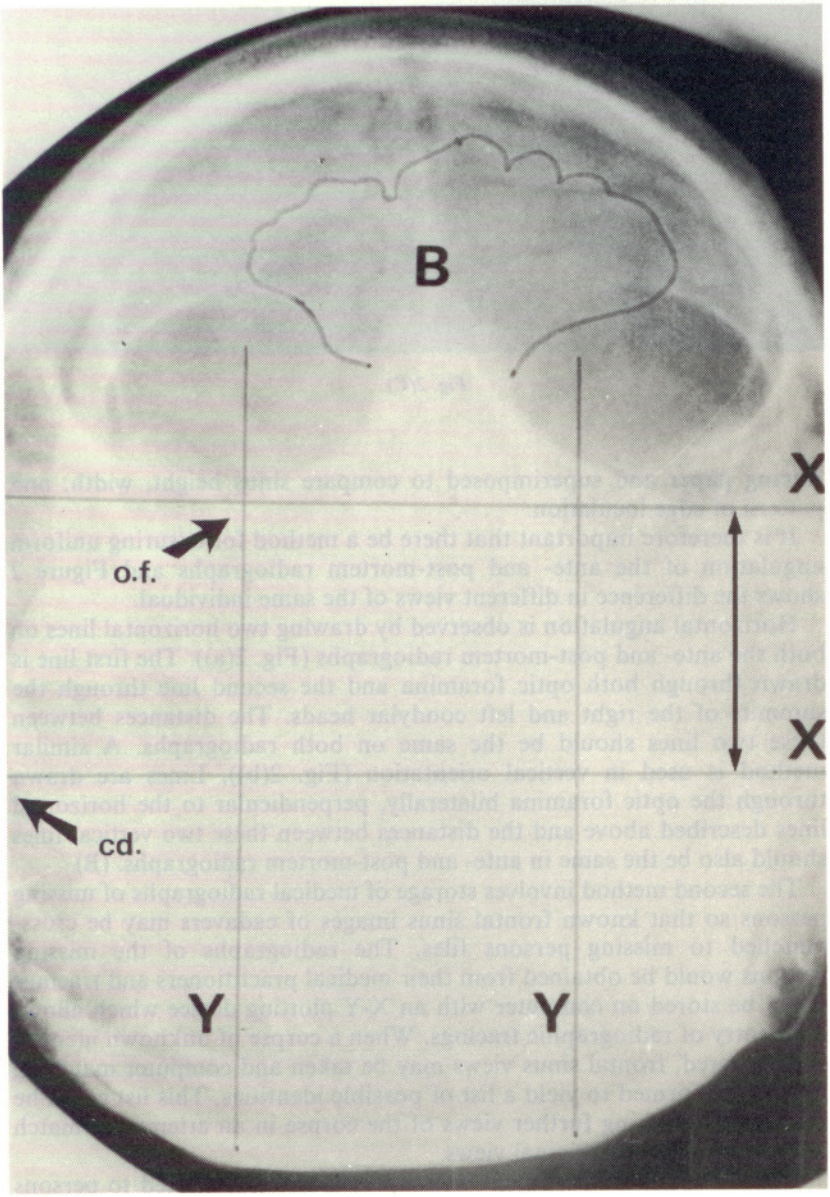

Fig. 2(B)

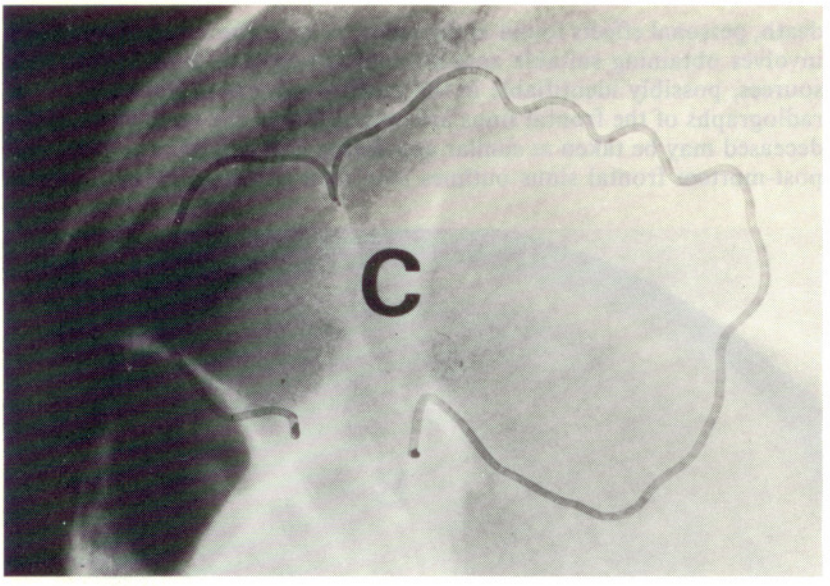

Fig. 2(C)

tracing paper and superimposed to compare sinus height, width, and pattern of edge loculation.

It is therefore important that there be a method for ensuring uniform angulation of the ante- and post-mortem radiographs and Figure 2 shows the difference in different views of the same individual.

Horizontal angulation is observed by drawing two horizontal lines on both the ante- and post-mortem radiographs (Fig. 2(a)). The first line is drawn through both optic foramina and the second line through the summits of the right and left condylar heads. The distances between these two lines should be the same on both radiographs. A similar method is used in vertical orientation (Fig. 2(b)). Lines are drawn through the optic foramina bilaterally, perpendicular to the horizontal lines described above and the distances between these two vertical lines should also be the same in ante- and post-mortem radiographs. (B)

The second method involves storage of medical radiographs of missing persons so that known frontal sinus images of cadavers may be cross-matched to missing persons files. The radiographs of the missing persons would be obtained from their medical practitioners and tracings could be stored on computer with an X-Y plotting device which allows data entry of radiographic tracings. When a corpse of unknown identity is discovered, frontal sinus views may be taken and computer matching may be performed to yield a list of possible identities. This list could be narrowed by taking further views of the corpse in an attempt to match angulation with the original views.

Use of the abovementioned techniques must be restricted to persons over the age of 20 years since frontal sinus size and outline may change

with growth. Further research is required to show the pattern and magnitude of these age-related alterations.

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Blood Group Substances in Human Salivary Glands

J. P. Roux*, and M. Marks*

**Faculty of Dentistry, University of Pretoria, P.O. Box 1266, Pretoria, 0001 South Africa*
***Department of Haematology, Ga-Rankuwa Hospital, P.O. Box 100, Medunsa, 0204 South Africa.*

Abstract

Blood group substances A, B and H present in the saliva of secretors are of great importance to the forensic odontologist, particularly with regard to bitemarks. The fluorescent antibody technique was utilized in determining the exact cytological localization of these water-soluble blood group substances in surgical specimens of mixed salivary glands. The results showed that the blood substances are water-soluble glycoproteins, mucus-bound and present only in the mucinous acini of the salivary glands examined. Serous acini and tissue from non-secretors stained negatively.

Introduction

Substances with antigenic properties in the various blood group systems, such as the ABO and Lewis systems, are present not only on the membranes of red blood cells but also occur as surface components of many tissue cells and in soluble form in a variety of secretions.[1]

The blood group substances found in body fluids are confined to those of the ABO blood group system and only about 75 per cent of all individuals secrete their corresponding blood group substances in their fluids.

Secretors usually produce A, B and H blood group substances in high concentrations in seminal fluid, saliva, vaginal secretion and gastric juices. In other body fluids such as sweat, tears and urine, the concentration is fairly low.[2]

Two forms of the A and B substances exist, namely those which are present on the surface of erythrocytes and tissue cells, and those found in body fluids and secretions. The former are alcohol-soluble glycolipids containing fatty acids, sphingosine and carbohydrate components. The secreted blood group substances are water-soluble glycoproteins composed of carbohydrate and peptide moieties.

The detection in the saliva of ABO blood group substances is of particular interest to the forensic odontologist. Many objects such as cigarette butts,[3] dental floss, tooth picks, chewing gum and dental appliances may contain enough traces of saliva for testing. Saliva stains may also be found on immovable objects at the site of a crime such as the floor, the ground and others. Human bites may be found in cheese, apples, on bottle caps and of particular interest to the forensic odontologist on human victims of assault. In all these cases it may be expected

that the amount of saliva surrounding a bite would vary considerably with the circumstances but in sexual murders bitemarks containing vast amounts of saliva may be found. However, if the biter is in a state of fear it may be expected that the mouth would be dry with relatively little saliva deposited. Although bitemarks may be found on a victim, it is possible that false reactions to blood group substances can be elicited.[4]

It is therefore essential that a control sample be taken from another part of the skin surface and a blood sample from the victim should also be grouped.

In spite of the complicated testing procedures there are occasions when it is useful to group saliva surrounding bitemarks. In the absence of a clearly defined and highly characteristic mark the grouping of saliva can be very valuable, particularly for the elimination of suspects. Furthermore, a mark which might appear to one observer to be an obvious bitemark might appear to others as an indecipherable pattern of injuries perhaps made by an object such as a sheriff's badge.[5] The absence of evidence of saliva from such a situation would provide some measure of corroboration for this view. It is therefore necessary when examining possible bitemarks that efforts be made to collect appropriate samples of saliva and if demonstrable, to do the necessary grouping tests.[6]

The use of blood group substances in medicolegal examinations is based on the fact that once a group is established in an individual it remains unchanged throughout his life.[7] The findings can only be used in eliminating a suspect rather than pointing conclusively to a certain individual.

Previous workers[8,9,10,11] have found relatively large amounts of water-soluble, mainly mucus-bound antigen in appropriate tissues of secretors which correspond with those found in saliva.

This study was undertaken to investigate histologically by means of immunofluorescence the distribution of blood group substances A and B in the sublingual and submandibular salivary glands of humans, and whether the blood group antigens were located in the serous or mucinous acini and in the duct epithelium.

Materials and Methods

1. Tissues containing the blood group substances

Twelve mixed salivary gland specimens from group A, group B and group AB individuals were obtained during surgical procedures where they were removed as a result of block dissection of the neck lymph nodes or in cases of open fractures of the mandible involving the floor of the mouth. Ten of these specimens were from secretors, and two from non-secretors (see below).

The immunofluorescence technique used in this study was a triple-layer staining method[12] using mixed salivary gland tissues. The underlying principle in the use of fluorescent antibody depends on the reaction between an antiserum tagged with fluorescein isocyanate and a specific antigen in a thin tissue section.

2. **Blood Group Antisera**

The first layer of antiserum used was mouse monoclonal anti-A and/or anti-B IgG reagent.* These are specific antibodies which are directed against the human red blood cell antigens A and B.

The second layer added was rabbit anti-mouse IgG reagent.** The lyophilized antiserum was reconstituted by the addition of 2,0ml of distilled water and stored frozen at –30°C in separate aliquots.

*Seraclone[R] Anti-A, -B. From Biotest Diagnostics, Frankfurt, West-Germany.
**Litton Bionetics Laboratory Products, Charleston.

The third and last layer used was goat anti-rabbit IgG conjugated with fluorescein isothiocyanate (FITC).* After reconstitution of the lyophilized conjugated specific antibody reagent by the addition of 2,0 ml of distilled water, the antiserum was stored at –30°C in separate aliquots. The antiserum was dilated in the ratio 1:10 with Evans blue when used.

3. **Preparation of the tissue sections**

The specimens were transferred to a cryostat cabinet at –20°C to –22°C where the tissues were frozen solid. After cutting sections of 6-7 μm thickness in the cryostat, they were transferred to glass slides and allowed to airdry for 30 minutes at room temperature. Sections were fixed in acetone for 10 minutes at room temperature followed by drying for 20 minutes in an incubator at 37°C. This procedure also facilitated the removal of the alcohol-soluble blood group substances.

The sections were stored at –30° in a deep freeze until further use.

4. **Determination of blood group and secretor status**

ABO blood group typing and determination of the secretor status of the patients from which the specimens were obtained, were performed by Lewis phenotyping of the red blood cells.[13,14] In two cases where the Lewis phenotype of the red blood cells was Le (a-b-), saliva was obtained from the patients for neutralization tests to determine the secretor status. Both cases proved to be secretors.

*Litton Bionetics Laboratory Products, Charleston.

5. **Staining of the sections**

One drop of the appropriate mouse monoclonal anti-A and/or anti-B reagent (i.e. anti-A for blood group A and anti-B for blood group B, and both, on different sections, for blood group AB) was applied to the sections.

The slides were immediately placed in a moist chamber and incubated at 37°C for 30 minutes. The antiserum was decanted and the slides were washed in phosphate-buffered saline (P.B.S.) at pH 7,2 for 15 minutes, using gentle agitation. The slides were wiped dry taking care to omit the area where the section was attached.

Each section was then covered with a few drops of the rabbit anti-mouse IgG reagent, put in a moist chamber, incubated at 37°C for 30 minutes, washed and dried as described.

Finally, each section was covered with a few drops of goat anti-rabbit IgG FITC and treated as before.

The sections were then mounted in P.B.S. with 10 per cent glycerol.

6. **Controls**

The following findings served to ensure that the yellow-green staining patterns were due to specific antigen-antibody reactions:

6.1 The areas did not fluoresce when treated with heterologous reagents. Thus, when in the first antibody layer anti-A was applied to a B tissue, or anti-B to an A tissue, no staining occurred.

6.2 The area under investigation did not fluoresce when treated with the labelled antiserum alone (i.e. goat anti-rabbit IgG FITC).

6.3 No staining was obtained in the 2 cases where tissue from non-secretors was used; the alcohol-soluble substances having been removed by the acetone fixation.

7. **Fluorescent microscopy and photography**

The fluorescent microscope used was a Reichert Polyvar with a standard Reichert source of ultraviolet light, equipped with a Wratten-2A barrier filter and a BG-12 excitor filter.

A 35 mm Fujichrome 400 daylight colour film was used to photograph the slides.

Results

Using the fluorescent antibody technique, 12 specimens of mixed salivary glands were examined from 10 secretors and 2 non-secretors Seven belonged to the blood group A, 3 to blood group B and 2 to blood group AB.

No difference was discovered in the occurrence and distribution of substances A and B in homologous subjects, or in AB individuals. Strong positive staining was produced with both anti-A and anti-B conjugate. This staining was confined to the mucinous cells, the serous cells being consistently negative* (Figs 1 and 2). Slight staining of the duct epithelium was also noted.

The antigen seems to pervade the mucus in the swollen cells of the mucinous acini, often obscuring the nucleus (Fig. 3) and in some cases overflowing across the cell membranes

The antigen was not present in the non-secretors used as controls, as well as those treated with heterologous reagents.

Discussion

The mouse monoclonal anti-A and anti-B antibodies reacted with the A and B substances, respectively, in the mucinous acini. The rabbit anti-

*As encountered by Szulman,[11] and experienced during this project as well, the histologic distinction between serous and mucinous acini in mixed salivary glands is not easily identified under the fluorescent microscope. Comparison of sections with adjoining ones stained with hematoxylin and eosin and examined under a light microscope helped in this respect.

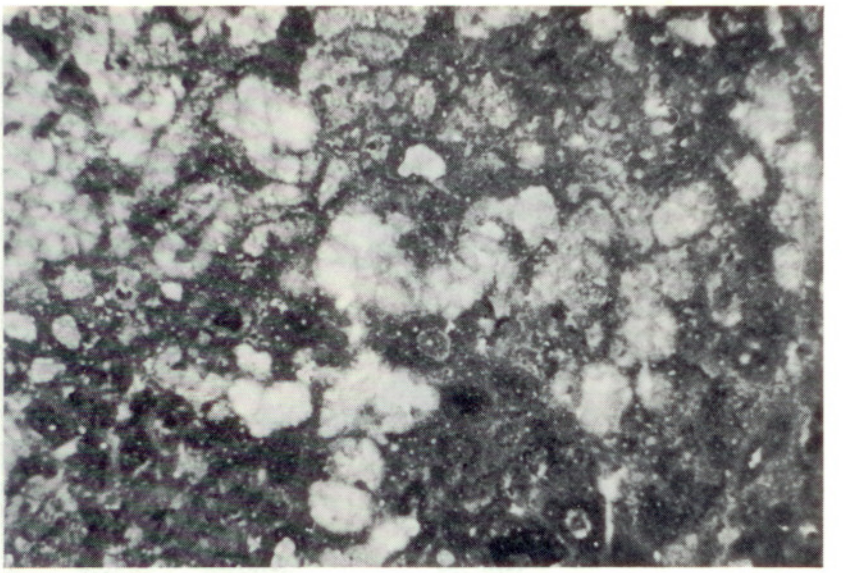

Fig. 1. Sublingual gland from a group AB secretor, stained with anti-A conjugate. Strong, positive staining of the mucinous acini, with no staining of the serous acini X125.

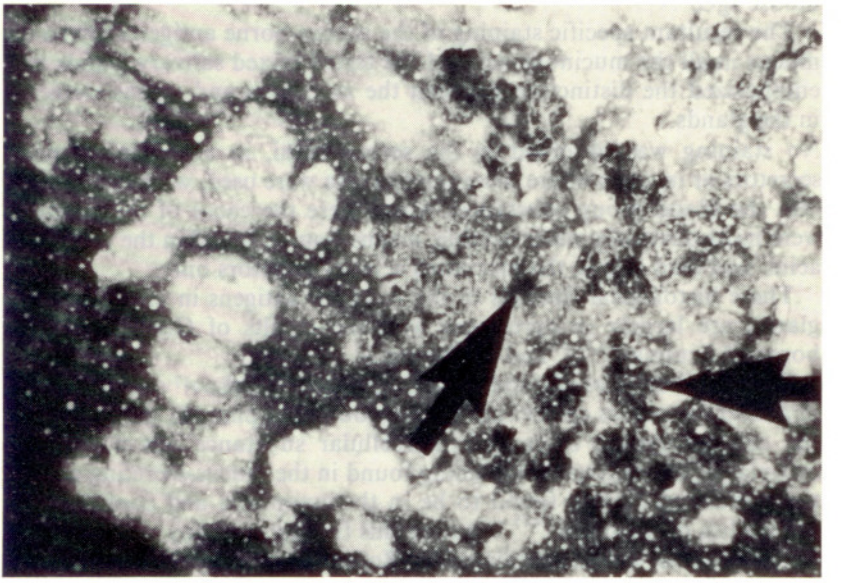

Fig. 2. Submandibular gland from a group A secretor, stained with anti-A conjugate. Unstained serous acini (Arrowed). X200.

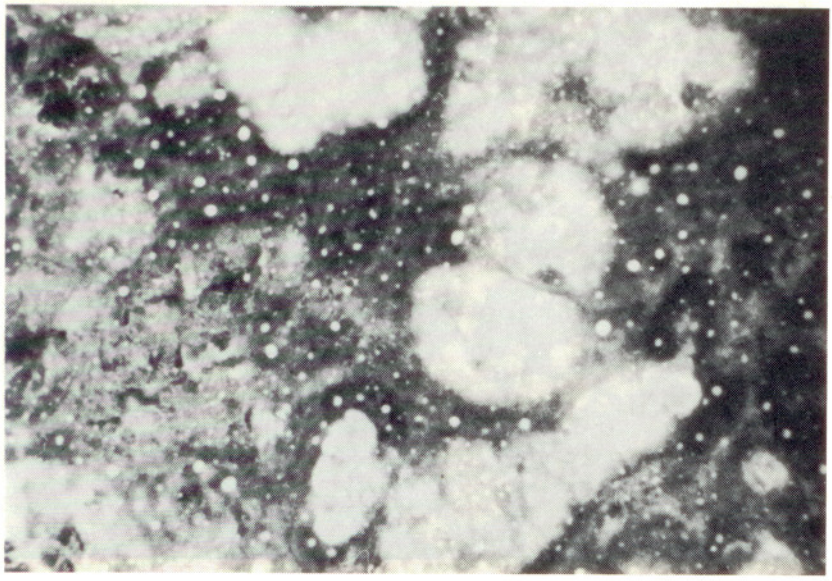

Fig. 3. Submandibular gland from a group B secretor, stained with anti-B conjugate. Positive staining mucinous acini. X300.

mouse reagent, in turn, reacted with the bound anti-A and/or anti-B and finally, the goat anti-rabbit FITC combined with the rabbit anti-mouse IgG.

The resultant specific staining of the mucus-borne antigen showed up mucus in all the mucinous acini of the tested mixed salivary glands and emphasized the distinction between the mucinous and serous elements in the glands.

No staining was observed in the serous acini, in any acini of non-secretors or in cases where heterologous sera were used, which confirmed that the blood group substances which were the cause of this interest, were most obviously water-soluble, mucus-bound, found in the mucinous acini, and then also in the mucinous acini of secretors only.

These histological findings of the A and B antigens in mixed salivary glands are largely in agreement with the results of Glynn and Holborow,[10] Szulman[11,15,16] and Holborow *et al.*[8]

Both Holborow *et al.*[8] and Dabelsteen and Fejerskov[17] stated that the stratified squamous epithelium of the mouth and pharynx stain either in the cell membrane or in the intercellular substance but not in the cytoplasm. In contrast, the staining found in the mucus-secreting cells in the present study was found to be in the cytoplasm and confirmed by the studies of Szulman.[11,15] Whether this finding is of any significance is not certain but it was postulated by Holborow *et al.*[8] that in the case of the stratified epithelia it may be possible that the blood group substances might have been absorbed from the secretions (saliva) bathing them. This absorption has been achieved experimentally by Holborow *et al.*[8]

The results of this project have shown that much can be gained by using the multiple layer "sandwich" technique, and that there is no more need for the production of strong, hyperimmune human sera conjugated with fluorescein. Commercially available bloodgrouping sera were used with great success.

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Radiologic Interpretation of Radiopaque Foreign Bodies of the Maxillofacial Region — A Review

R. E. Wood, A. M. P. Harris and C. J. Nortjé

Department of Maxillo-facial Radiology, Faculty of Dentistry, University of Stellenbosch, Private Bag X1, Tygerberg 7505, Republic of South Africa

Abstract

The various methods of evaluation of radiopaque foreign bodies of the maxillofacial region are reviewed. Emphasis is placed on a systematic approach to the successful interpretation of images rather than radiographic technical methods. The weakness of panoramic radiographs in the localization of foreign bodies of this area is stressed.

Key words: Radiology, foreign bodies, forensics.

Introduction

Gibilisco (1985) stated that more foreign bodies are discovered in the face than in any other region of the body.[1] Foreign objects may present as radiolucencies or radiopacities but the latter are observed more frequently.[2] A radiolucent body, on the other hand, will most probably be overlooked unless it is positioned within an area more radiopaque than itself.

From a radiological point of view, there are two properties of any suspected foreign body, its nature and its position, which should be determined prior to surgical exploration. Amalgam alloy is the most common radiopaque foreign body in the facial region although dental instruments and gunshot are also frequent findings.[1]

There is a tendency for the inexperienced clinician to visualize radiographs as being three-dimensional representations of objects when in fact they are not. Because they are two-dimensional a single film cannot yield an interpretation of the location of objects;[3] which is commonly seen in the case of the panoramic radiograph. Hudson, in 1957, developed the first commercially viable panoramic radiography machine and he stated that panoramic dental radiography was not a substitute for intra-oral radiography but rather a device suited to mass screening of large numbers of patients.[4] Despite this disclaimer, panoramic radiography has continued to enjoy widespread acceptance, particularly for the radiography of trauma patients,[5] even though the resolution is half that of intraoral films.[6] The reason for this probably stems from its convenience rather than image quality. There are, however, definite occasions when panoramic radiographs should be used with caution, one of which is the radiographic examination of patients with suspected foreign bodies.

In our opinion when an inexperienced clinician is faced with a radiopacity, he tends to resort to guesswork rather than a process of systematic interpretation. The interpretation system used by the authors is one of describing the lesion, then from these observations deducing its structure, and finally determining its precise location within the patient.

I. The nature of a radiopacity

Radiopacities of the maxillo-facial region are caused by four types of materials: bone, calcification (either metastatic or dystrophic), dental

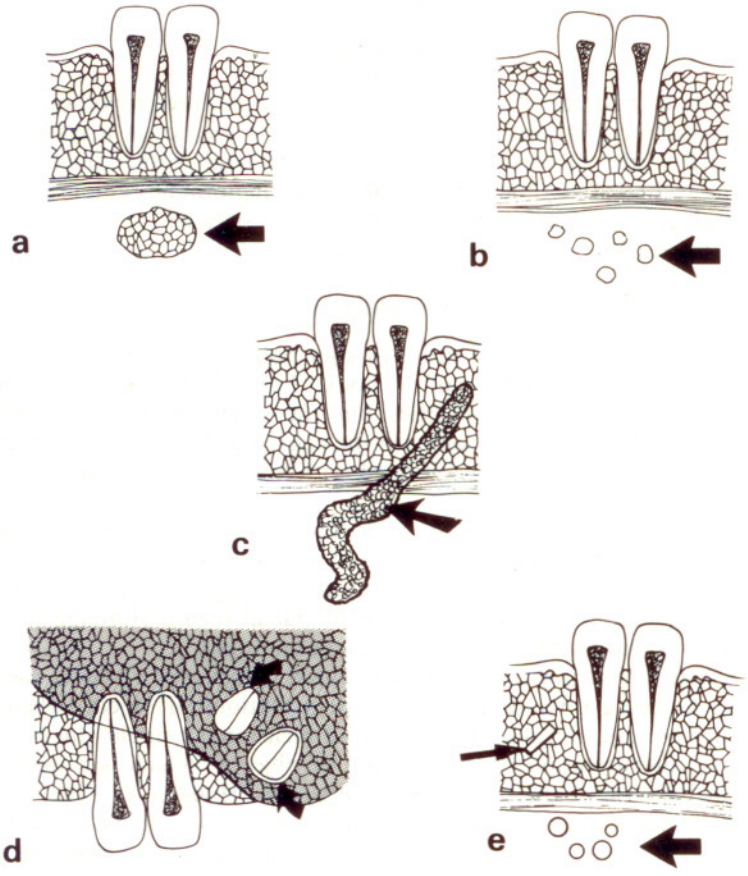

Fig. 1. Bone as a foreign body (a) exhibits a well defined border, trabeculae and marrow spaces and density similar to that of adjacent bone. Dystrophic calcification (b) is usually well-defined, has no marrow spaces or trabeculae, may have a smooth or roughened border and be of greater or lesser density than adjacent bone. Metastatic calcification (c) may be widespread, positioned in some form of pattern such as in tissue planes or vasculature, is patternless and less dense than bone. Teeth as foreign bodies (d) should be of similar radiopacity as other teeth visible on the film, should be tooth shaped, and if in bone have a ligament space and lamina dura. Metallic foreign bodies (e) are much more opaque than anatomic structures, may be of any shape and pattern and be located anywhere.

structures, or true foreign body, each of which have their own particular radiologic features (Fig. 1).

Bone fragments may present as foreign bodies and have the typical radiologic appearance of normal trabecular bone. Bone fragments should therefore have trabeculae, marrow spaces and be of a density similar to adjacent normal bone.

Pathologic calcifications may also be confused with foreign bodies. Dystrophic calcification should be generally well defined, have no marrow spaces or trabeculae, little structural organization, and be of a density less than or equal to adjacent normal bone. The margins of dystrophic calcification may be smooth or roughened and areas of dystrophic calcification may be of any size. Metastatic calcifications are generally multiple and often widespread. They are usually positioned in some pattern such as in vessels or in tissue planes. They are radiologically patternless with ill-defined margins and no evidence of trabeculae or marrow spaces.

Teeth may also become foreign bodies, especially in cases of trauma. If a foreign body is suspected of being a tooth it must display the same density as adjacent teeth or teeth present on other radiographs. It should also be of such a shape as to be recognizable as a whole or part of a tooth. The tooth or fragment should have a pulpchamber or root canal within its confines and if it is within bone, it must have a periodontal ligament shadow.

True foreign bodies are materials that originate from outside the body which find their way into the tissues of the region, and may therefore be positioned almost anywhere. They may be unrecognizable if they are radiolucent and if they are radiopaque they may or may not be recognizable as dental instruments, or projectiles. They are often more opaque than even tooth enamel which helps to differentiate them from tooth fragments.

II. Locating the Foreign Body

When the nature of the foreign body is known, the next step in systematic interpretation is to determine its position, which is necessary prior to surgical exploration. This may be accomplished by: relation of the foreign body to known anatomic landmarks using a single film; relative magnification of the object relative to the film plane; use of the principle of parallax by utilizing tube shift techniques (horizontal or vertical buccal object rule); use of the principle of two views at right angles to each other; and via sophisticated methods such as tomography or computer assisted tomography. In this paper only examples of metallic foreign bodies will be presented but in fact the same techniques may be used for any object.[7,8]

The above-mentioned radiographic projections must often be customized in order to meet the unique requirements of each case.

Technique 1. Relating the foreign body to known anatomic structures using a single film

This is one of the least reliable of the techniques because of the two

dimensional nature of radiographic images. Its successful use is predicated on a thorough knowledge of radiographic anatomy and the use of selected techniques involving small tissue volumes. It is particularly useful in confirming the presence of foreign bodies whose general location has been suggested from non-radiographic means, such as history, signs, or symptoms. Examples of this technique are investigation of foreign bodies of the buccal soft tissues by radiographing the soft tissue of the cheek and similarly in the floor of the mouth (Fig. 2).

Technique 2. Determination of position by relative magnification of the object relative to the film plane.

This is another technique which is not completely reliable but it may be useful where only a single view is available. In order to be successful it is important to have an estimate of the true size of the object in question. It is vital to know the method by which the projection was

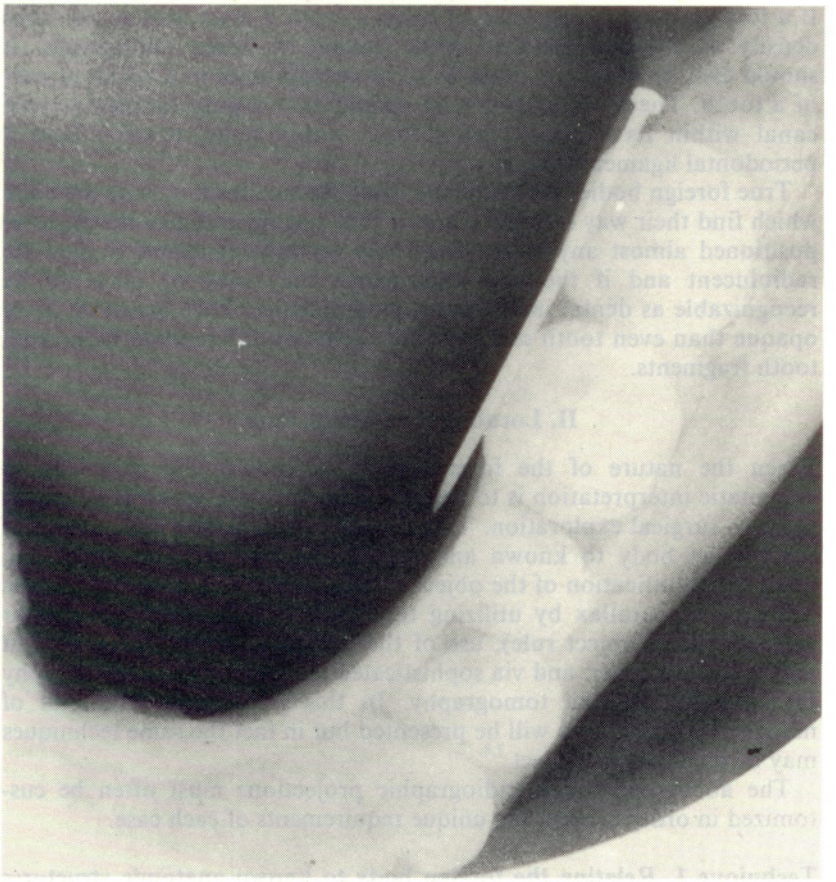

Fig. 2. Occlusal radiograph of metallic object in floor of mouth. This radiograph of a small tissue volume aids in narrowing down the area to be searched.

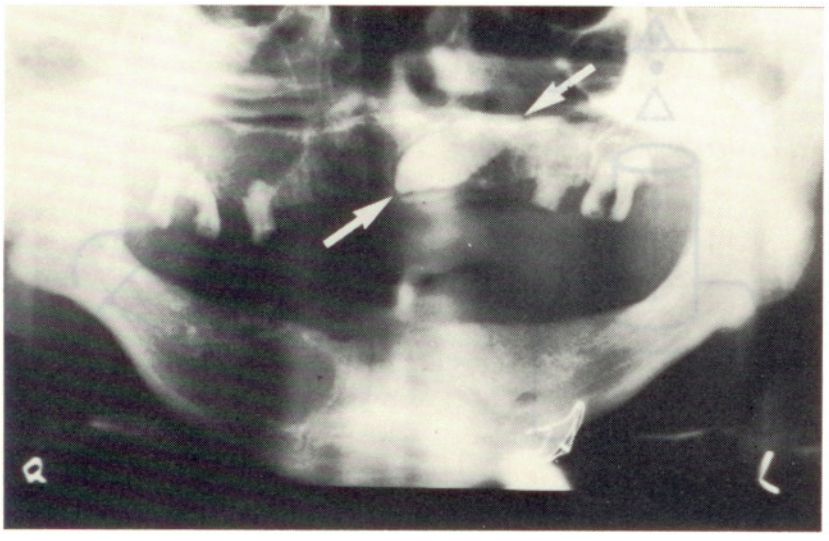

Fig. 3. Panoramic radiograph showing grossly enlarged image of cuspid in maxilla. This enlargement demonstrates its palatal position.

made including exact film placement and beam angulation relative to the object and film plane. Three clinical situations where it is useful present themselves: determination of the location of the crown in transversely impacted third molars, evaluation of impacted cuspid teeth (Fig. 3) and evaluation of the relative position of shotgun pellets if their size is known. It is not suggested that this method be used exclusively, but it should serve as a way of selecting appropriate additional radiographs.

Technique 3. Parallax principles as an aid in position determination.

In theory this is a reliable method to determine object position but in practice it is not as successful. Part of the reason for this is that the person who interprets the radiographs must also expose them. It is therefore suggested that the first projection be taken, processed and examined. Following this, a second view (done with a tube shift of known direction) allows the investigator to select the direction and amount of tube shift to maximize separation of the shadows of the objects being radiographed from a reliable landmark of known location. The process may be performed using horizontal or vertical tube shifts (Figs 4a and 4b). It may also be performed with panoramic and periapical film combination although this is difficult for even the experienced clinician because of the small tube shift between the two (Fig. 4c). In order to localize a single object the "movement" between films must be compared to a known landmark. At times there is no landmark available (for example in the edentulous patient) and to overcome this, a wax record-block with a metallic object whose precise location is known may be placed in the patient's mouth. Two radiographs

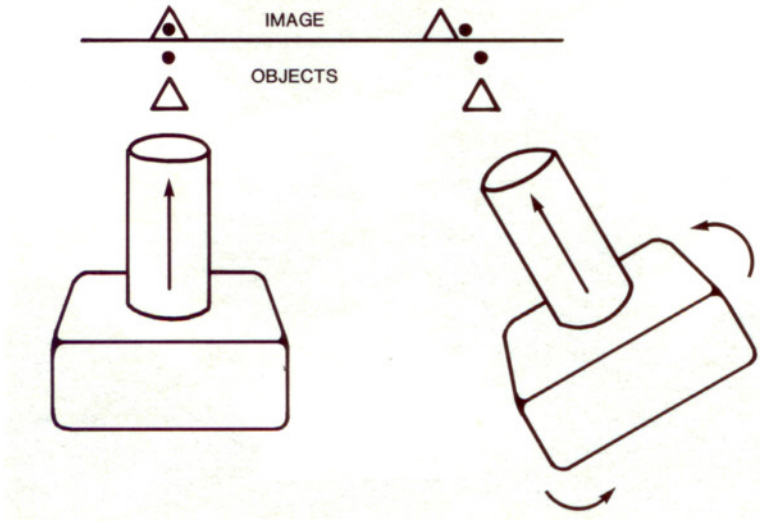

Fig. 4(A)

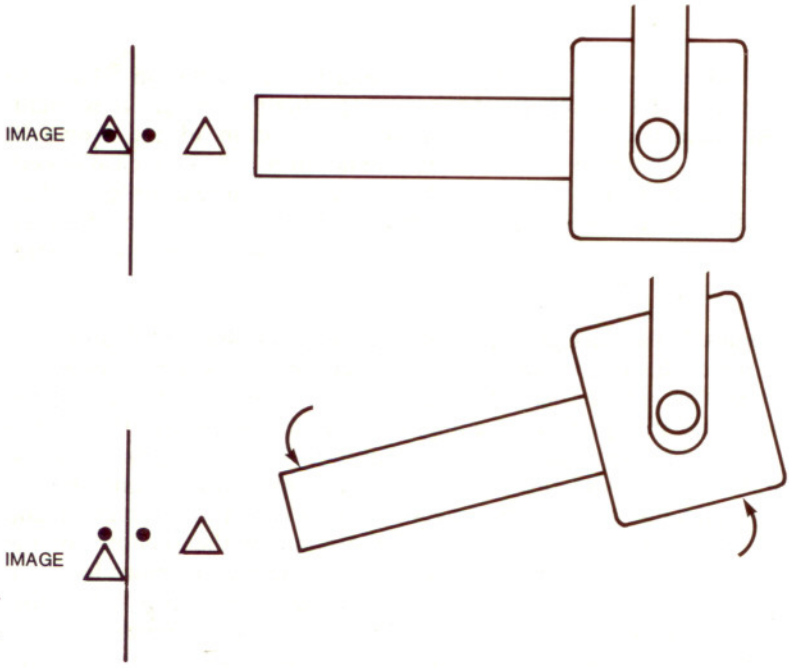

Fig. 4(B)

Fig. 4. Use of horizontal tube shift (a) and vertical tube shift (b) for the localization of a foreign object. Use of a combination of panoramic and periapical radiographs (c) is possible since panoramic radiographs are taken with negative angles (upward directed beam from the lingual) which causes the image of any object on the lingual aspect to be positioned superiorly on the panoramic view.

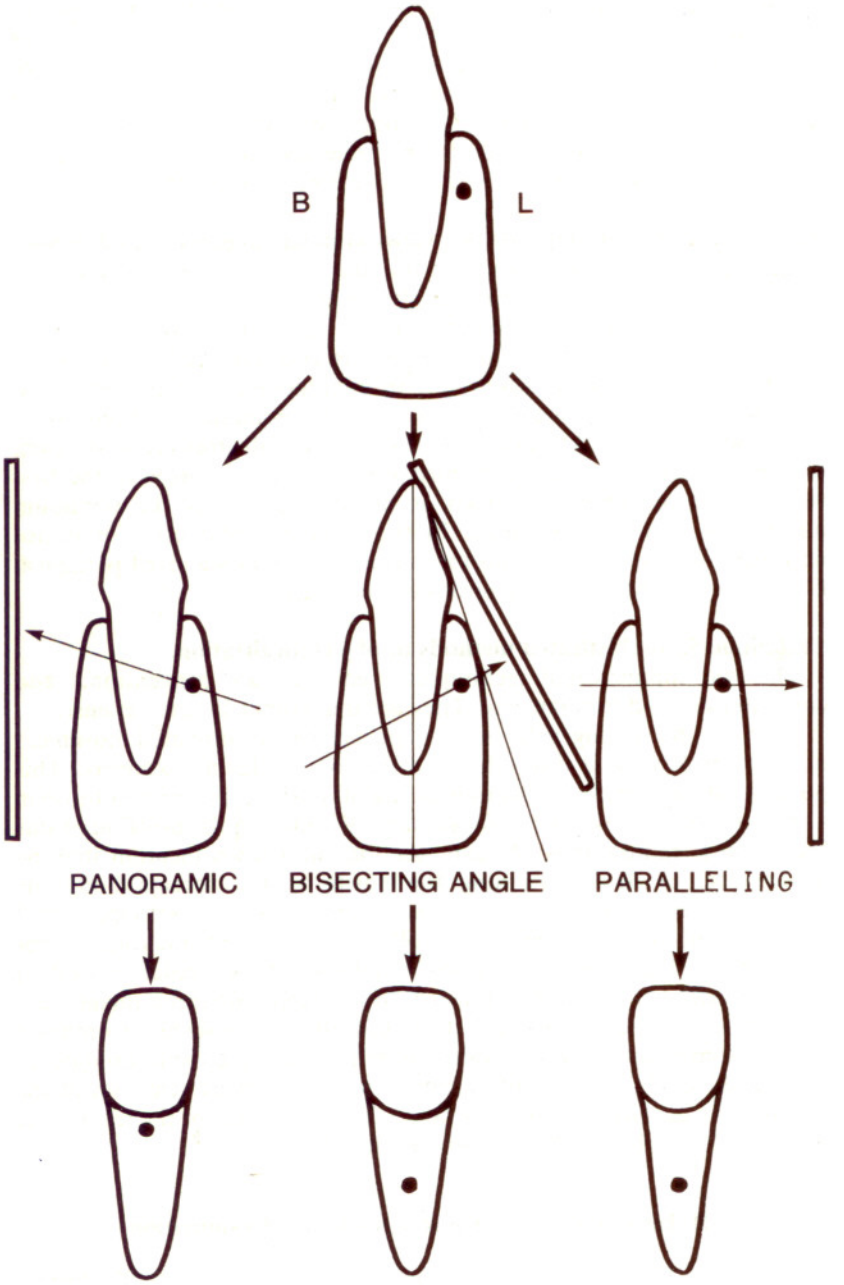

Fig. 4(C)

taken with a tube shift may be made and the relative position of the foreign body to the landmark can be deduced.

There are several rules for determining the localization buccally or lingually of a foreign object and the authors find the rule of SLOB (same lingual opposite buccal) most convenient. This means that if the image of the object moves the same way as the tube moves as seen on the second image it is lingual.[9] If it moves opposite to the tube shift it is buccally positioned. It should also be remembered that objects further from the film plane move more than close objects (Figs. 4a and 4b).

Technique 4. The principle of two views at ninety degrees to each other

This is without doubt the most reliable method of localization and requires at least two views at ninety degrees to one another.[7]

The views may be skull views as seen in Fig. 6 or true occlusal views. While in the case of the mandible this is accomplished by using the standard mandibular occlusal view in conjunction with the intra-oral periapical view, the process is not as easy in the maxilla as it requires a vertex occlusal view. At times two views at ninety degrees are insufficient for positive localization and a third view at ninety degrees to the first two is required (Fig. 5). "Two at ninety" represents the minimum number of views to be taken. In the private dental office it is not necessary to stock special film for occlusal views as standard periapical films may be used if they are positioned carefully.

Technique 5. Sophisticated methods of object localization.

The two sophisticated techniques used most are conventional and computer assisted tomography. They are unfortunately also expensive.

Conventional tomography involves purposeful co-ordinated movement of the tube head and film relative to the object during exposure. This purposeful movement results in blurring of all structures except those in a specific zone known as the focal plane. By altering the position of this focal plane, radiographs of tissue sections of known location may be made (Fig. 6). In determining the position of a foreign body the radiologist selects a series of sections or "cuts" which he anticipates will cross the path of the foreign body. Following processing, the tomographs are compared and the precise position deduced. A similar procedure may be used in computer assisted tomography wherein images are reconstructed by computer and displayed on a video screen. Computer assisted tomography has the added benefit of allowing the radiologist to see soft tissue, bone and radiolucent foreign bodies by selection of the appropriate density and contrast range. Further details of the techniques have been described by Bryan[10] and Pullan.[11]

III. Importance of a Thorough Clinical Examination

Even with adequate numbers and types of radiographs radiology plays a limited role in identification and localization of foreign bodies of the maxillofacial region. Radiographs should only serve to confirm clinical findings. History of the traumatic incident (Fig. 7), knowledge of

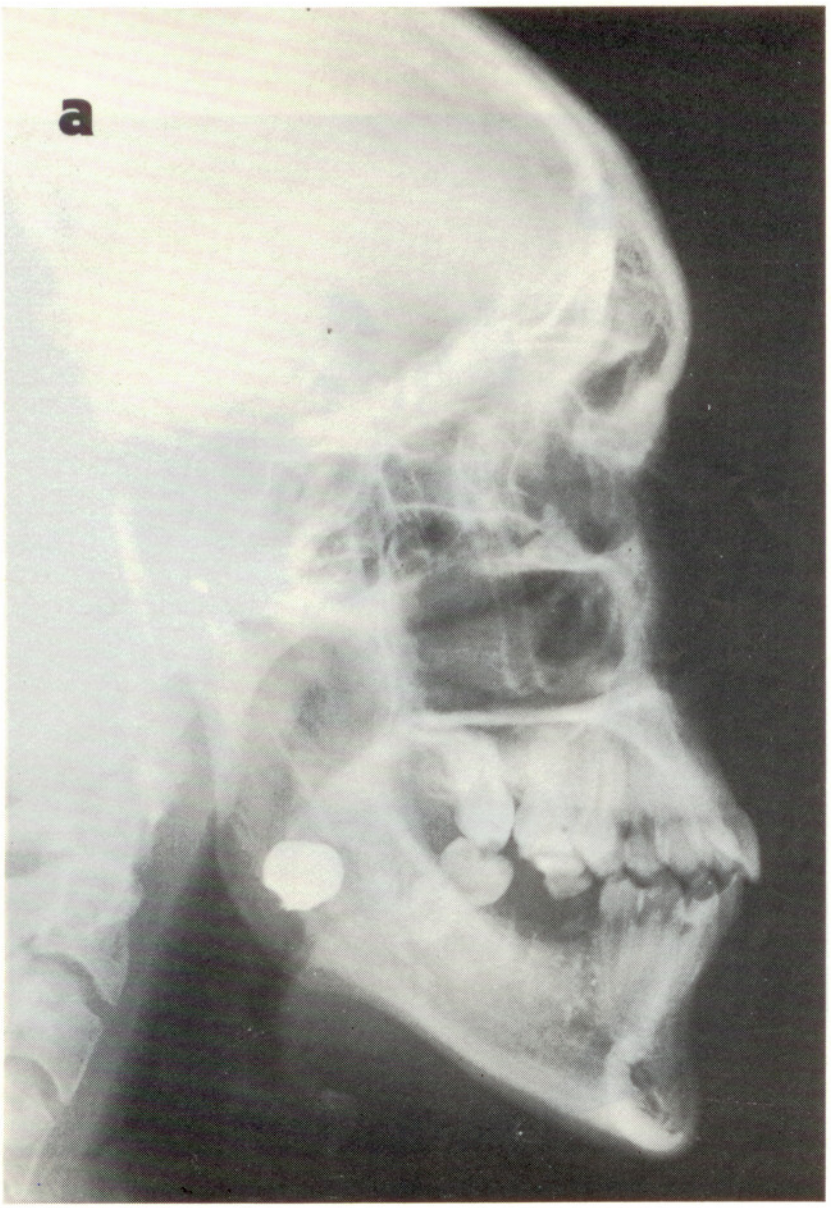

Fig. 5(A)

Fig. 5. Radiographs of gunshot victim. The posteroanterior view and lateral projections taken together (A and B) are inadequate for precise object localization until supplemented with a basal view (C).

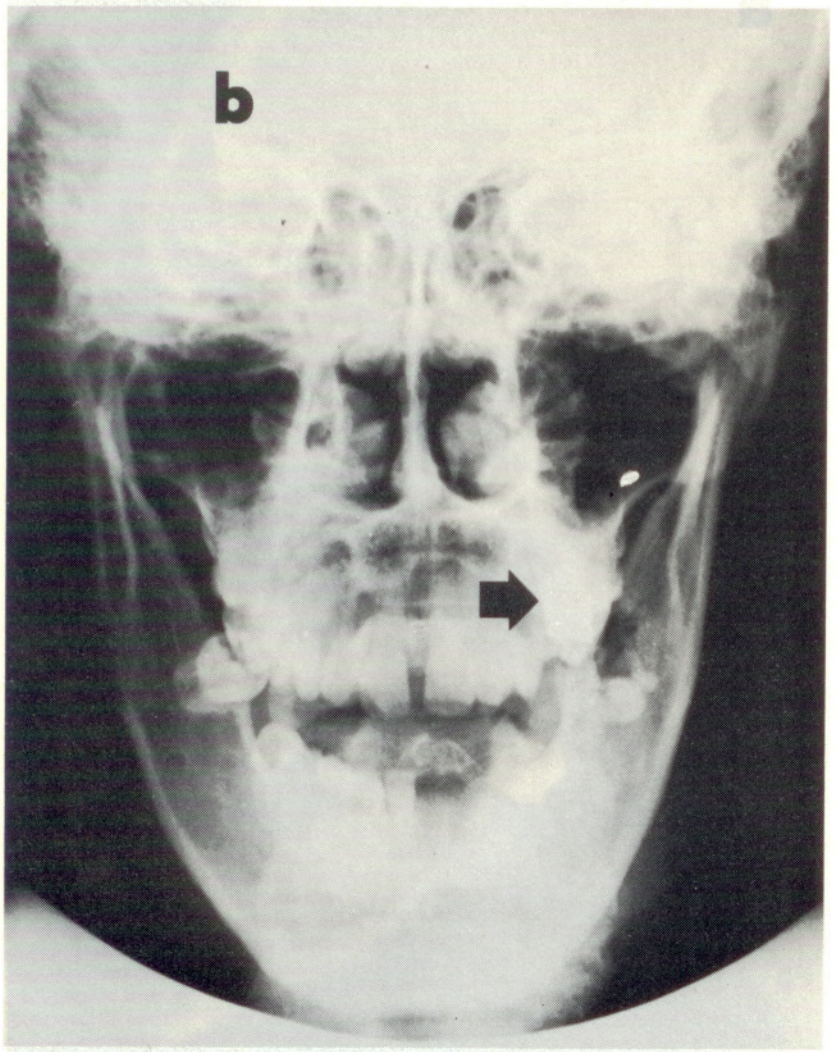

Fig. 5(B)

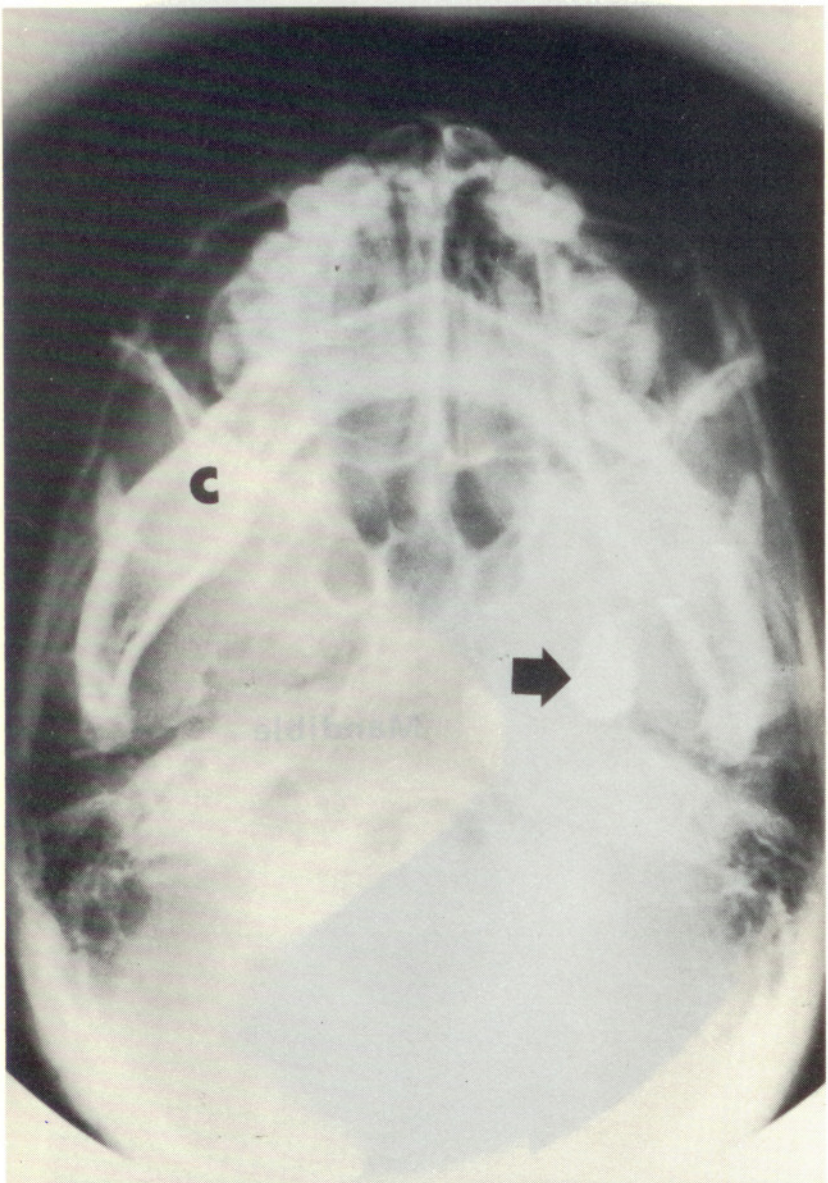

Fig. 5(C)

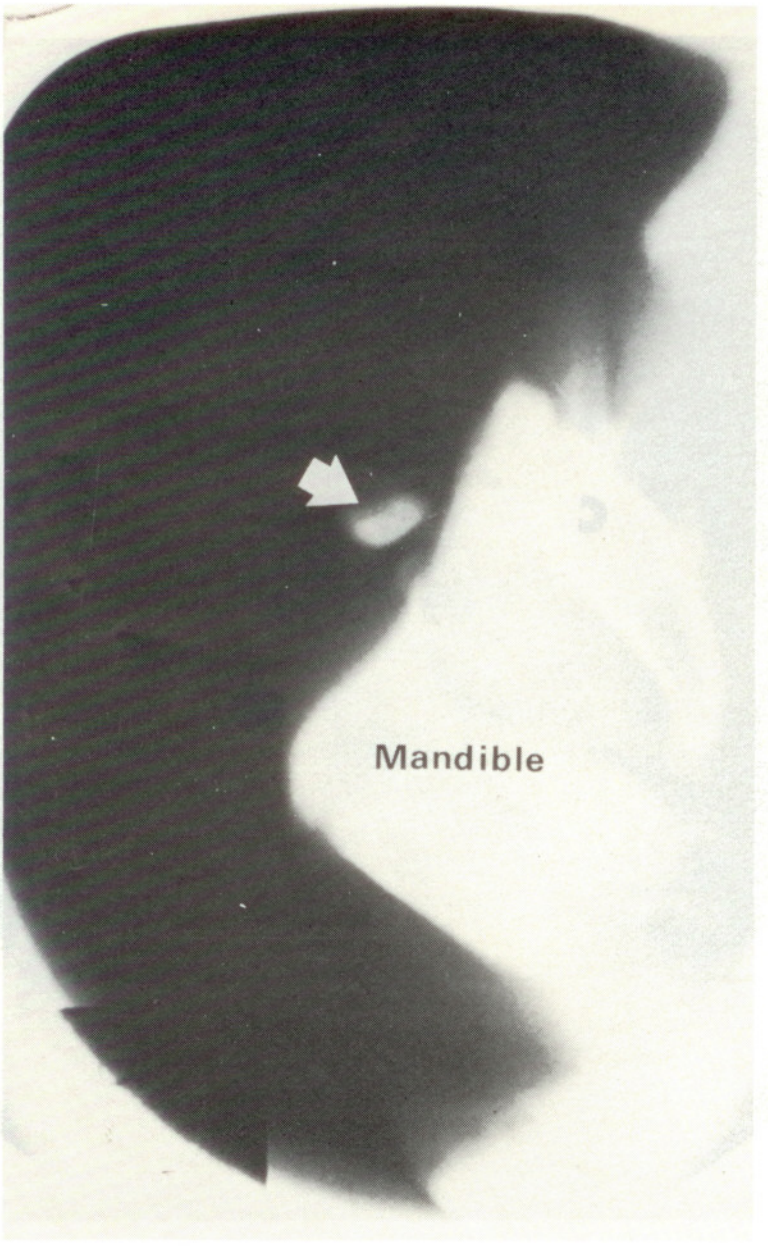

Fig. 6. Tomographic sections through foreign object — note blurring of other structures.

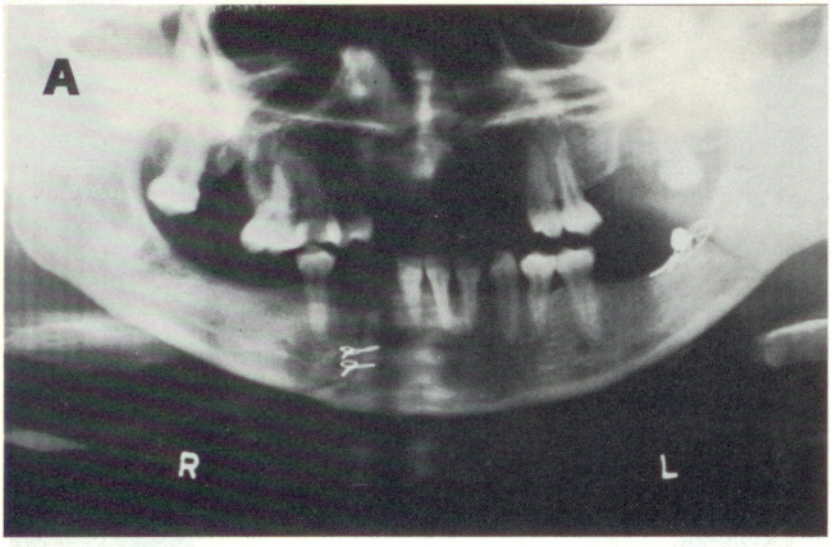

Fig. 7(A)

Fig. 7. Note the paucity of pellets on the panoramic radiograph of this patient who was shot ten years ago. PA skull and chest radiographs (B and C) show the true extent of the injury.

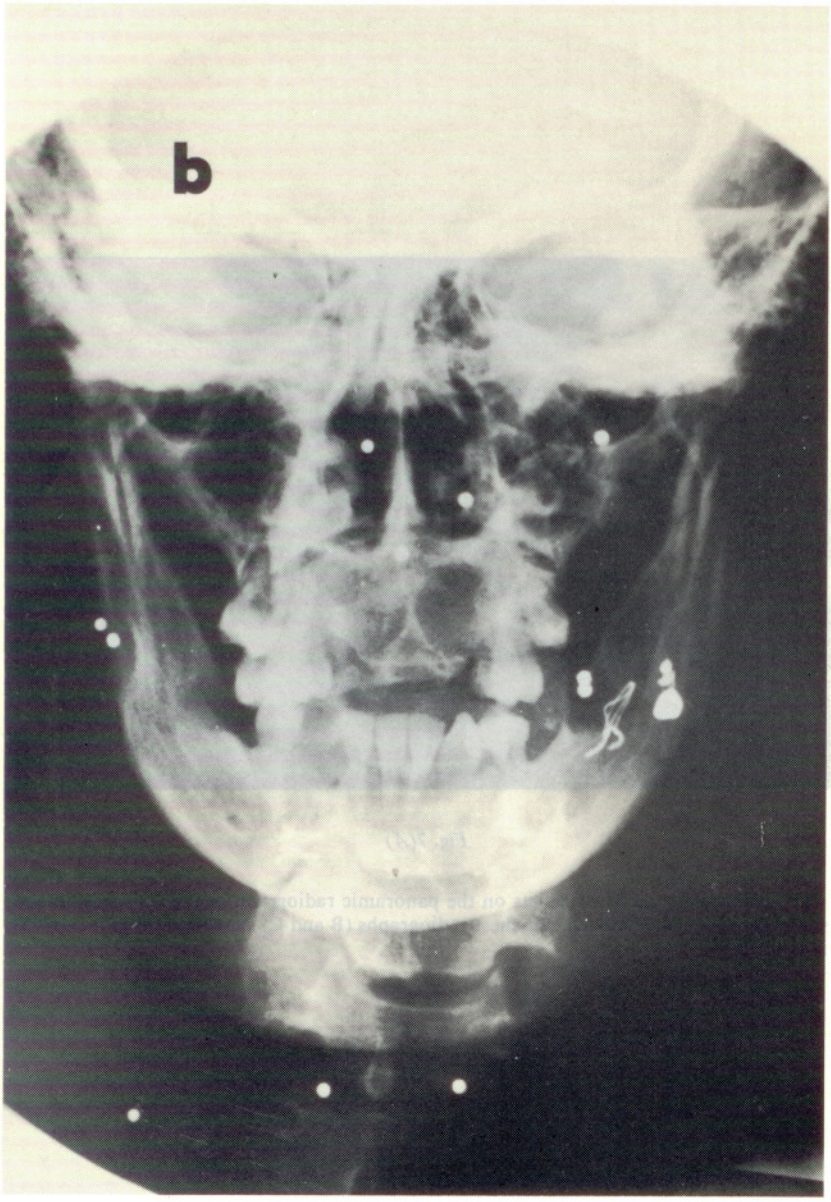

Fig. 7(B)

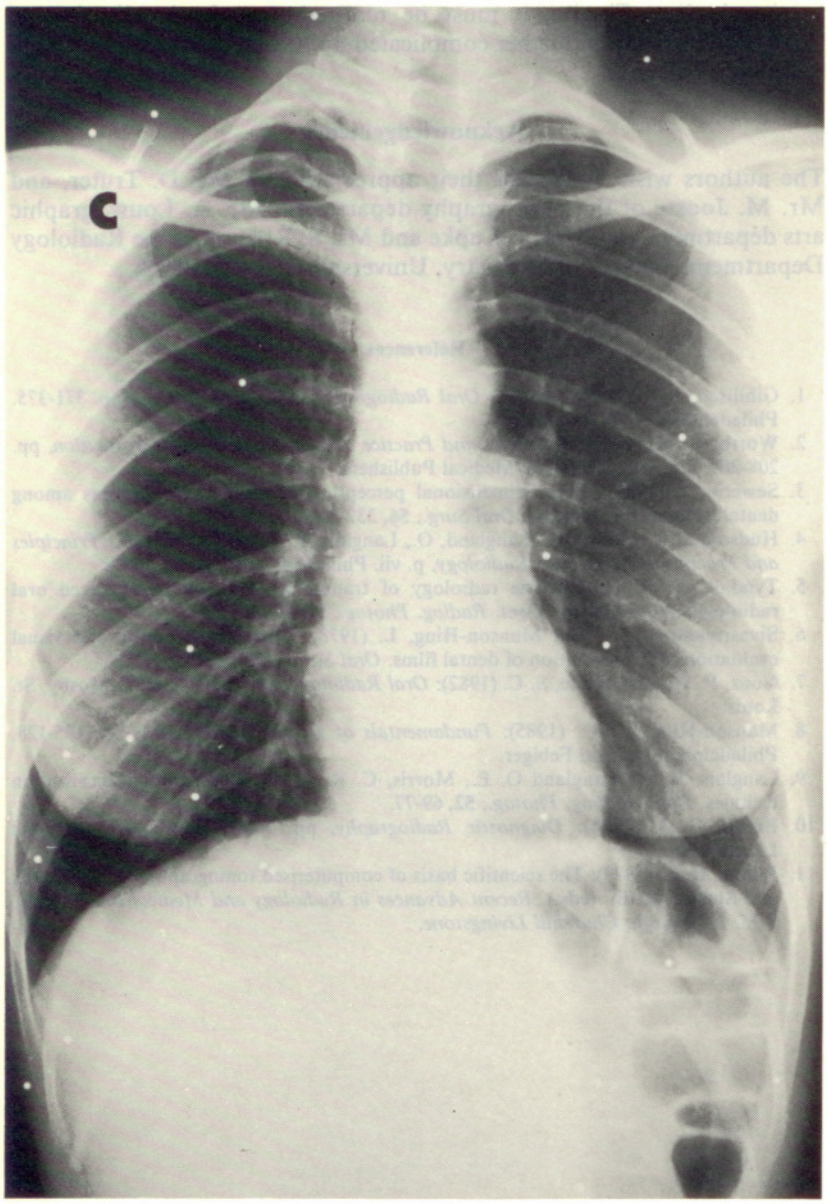

Fig. 7(C)

entrance wound sites and probable foreign body trajectory are particularly relevant. Clinical examination should also include the use of bimanual palpation for determination of the presence of soft tissue foreign bodies. Finally, it must be realized that the localization of foreign bodies may be further complicated by their subsequent movement within the body.

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