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IMPROVEMENTS ON THE TECHNIQUE FOR THE PRODUCTION OF WAX MODELS OF BIOLOGICAL STRUCTURES

by

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INTRODUCTION

A method of producing magnified three dimensional models of microscopic specimens was described by *Born* in 1883. In essence, his method involved tracing of the particular structures from serial microscopic sections onto wax plates, the thickness of which was equal to the thickness of the histological section multiplied by the desired magnification. The plates were then cut along the traced outlines and placed on top of one another in the proper sequence. Such models, by melting the plates together, represent magnified three dimensional reproductions of the original specimens.

The method had many shortcomings and was subsequently improved (*Born*, 1888; *Born & Peter*, 1898; *Peter*, 1906; *Peter*, 1927). A major problem in wax reconstruction has been to furnish the specimen with reference points, in such a way that the wax plates could be precisely orientated one to another. If placed outside the tissues the reference points tended to be displaced during the laboratory processing and placing them within the tissues often caused damage to the structures of interest.

In recent studies of the three dimensional configuration of the epithelium-connective tissue boundary (*Löe & Karring*, 1967; 1969; *Karring & Löe*, 1970) the authors have improved the technique for the fabrication of wax

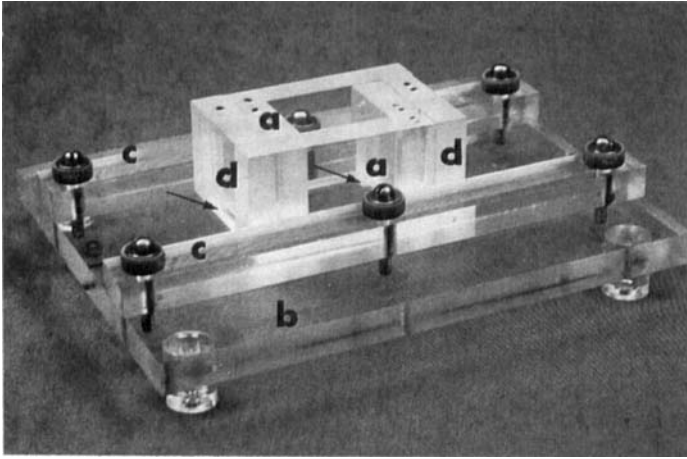


Fig. 1. Embedding chamber for the production of paraffin blocks securing a proper relationship between the reference points and the plane of sectioning. A right angled box is formed upon assembly of the two acrylic L-pieces (a) on the bottom plate (b). Laterally they are supported by two parallel lists (c). Slits (arrows) at the short branches are closed by two pieces of acrylic (d). The midline of the bottom plate is indicated by a black line (e).

reconstructions, making it less time consuming and also facilitating the production of proper reference points within the tissues in a controlled manner. This paper describes the essential features of the method.

THE METHOD

Immediately after biopsy the surface of the tissue specimen which will later on be furnished with reference points, is painted with a slightly heated mixture of Indian ink and 25 per cent gelatine solution (1:4). The painting is performed under a dissecting microscope and the specimen is inserted into the fixative where the mixture precipitates as a black surface coat. After fixation the specimen is made subject to paraffin infiltration.

To ensure that, when the wax block is placed on the block holder of the microtome, the angle between the plane of sectioning and the reference points is exactly 90° , paraffin embedding is performed in a special chamber (Fig. 1). This consists of two L-shaped pieces of transparent acrylic (a) which upon assembly on a bottom plate (b) forms a right angled box (Fig. 1). The slits at the junction between the bottom plate and the short branches of the L-pieces (Fig. 1, arrows) make it possible to utilize the same arrangement for the production of reference points (see later). During the embedding procedure, however, the slits are closed by two pieces of acrylic of a size

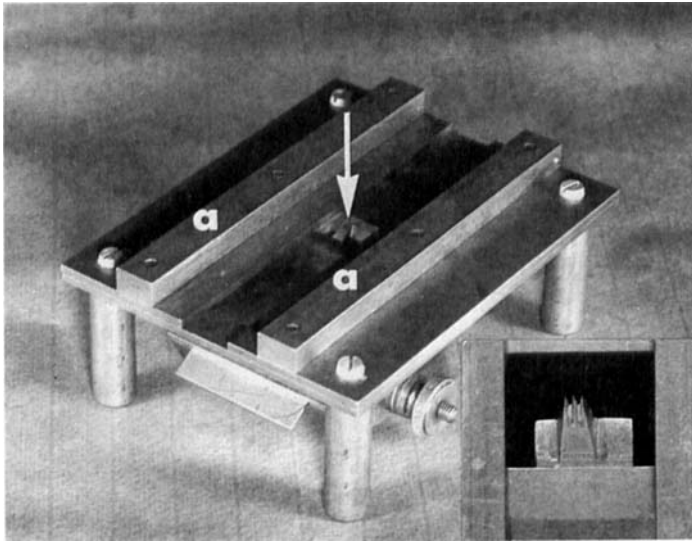


Fig. 2. Notching apparatus for the production of reference points. The metal table is equipped with an adjustable knife with three V-shaped cutting edges (arrow, inset). When brought against the knife, the paraffin block with the L-pieces is guided by the two parallel lists (a).

equal to that of the short branches (d). The embedding chamber is supported laterally by two parallel lists (c) and the midline of the bottom plate is indicated by a black line at its subsurface (e).

Prior to the embedding of the specimen the walls and floor of the chamber are painted with a mixture of glycerol and absolute alcohol (1:1) and the apparatus is heated in the paraffin oven for approximately $\frac{1}{2}$ hour.

The apparatus is next placed in a shallow dish and the entire chamber filled with paraffin. Orientated according to the desired plane of cutting, the specimen is placed at the black line in such a way that the surface of the tissue, into which the reference points are to be made, faces downwards. Cooling is provided by ample amounts of ice water in the dish. The resulting shrinkage of the paraffin block is compensated for by keeping the surface of the paraffin melted by means of a hot spatula during the cooling and by adding molten paraffin until the chamber is completely filled.

The production of reference points. After the paraffin has hardened the external acrylic pieces which closed the openings at the short ends of the embedding chamber (Fig. 1 d) are removed and the paraffin block, still surrounded by the L-pieces, is transferred to the notching apparatus for production of reference points.

The notching apparatus (Fig. 2) consists of a metal table equipped with a

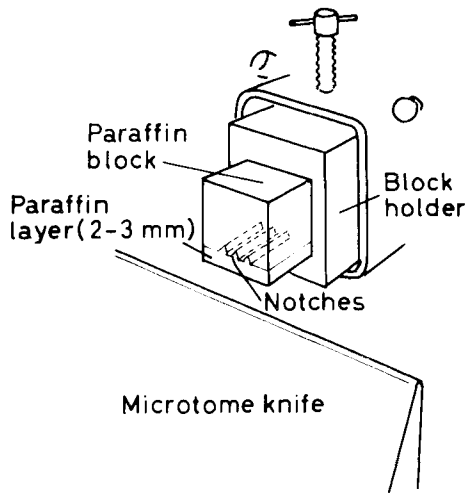


Fig. 3. Diagram showing the paraffin block, furnished with reference points (notches), properly mounted in the microtome.

knife with 3 V-shaped cutting edges (Fig. 2, arrow, inset) which prior to use are adjusted at a level $\frac{1}{4}$ – $\frac{1}{2}$ mm above the table. Guided by the two parallel lists (Fig. 2a) the wax block, with the L-pieces, is brought against the knife and three parallel grooves are produced in the lower surface of the wax block and the adjacent part of the specimen. Since this surface of the tissue was coated with Indian ink-gelatine it is possible to check in a dissecting microscope whether or not the knife has reached into the specimen. The height of the knife is adjusted until grooves of appropriate depth are produced in the specimen.

The block is then removed from the L-pieces and the notched surface is covered with a layer of paraffin 2–3 mm thick (Fig. 3). Finally, the excess paraffin on the adjoining surfaces is removed and the block is ready for mounting on the block holder.

The mounting of the paraffin-block. To secure the proper orientation of the block the base of the block holder is covered with a 3–4 mm thick layer of paraffin which, after setting, is levelled by the microtome knife. The block is then placed on the block holder with the notched surface of the specimen right angled to the cutting edge of the knife (Fig. 3). A hot needle is inserted at intervals along the interface between the block and block holder. This ensures that the fusion of the block to the block holder is attained with no distortion of this relationship one to another. Finally, melted paraffin is added to the site of junction between the block and the block holder.

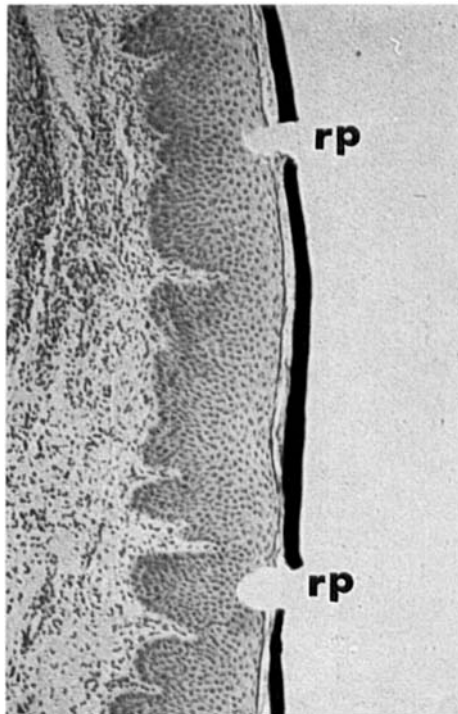


Fig. 4. Histological section of gingiva furnished with reference points (rp) in the epithelium. The surface is covered by a black coat of indian ink-gelatine.

The production of paper replicas. The serial sections selected for reproduction should be of good quality and have appropriate reference points (Fig. 4). The selected part of the tissue, including the reference points, is projected on to white paper at the desired magnification and the outlines are drawn on the paper. In order to prevent inversion, the section is projected via a mirror at an angle of 45° on to the sheet of paper placed on a horizontal glass plate (Fig. 5).

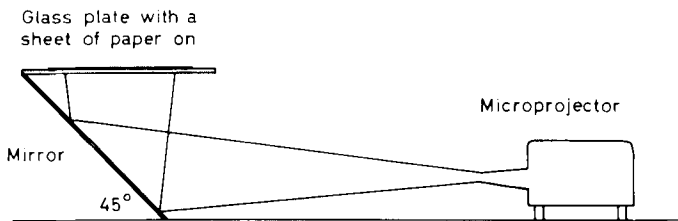


Fig. 5. Diagram illustrating the projection of histological sections on to sheets of paper placed on a horizontal glass plate.

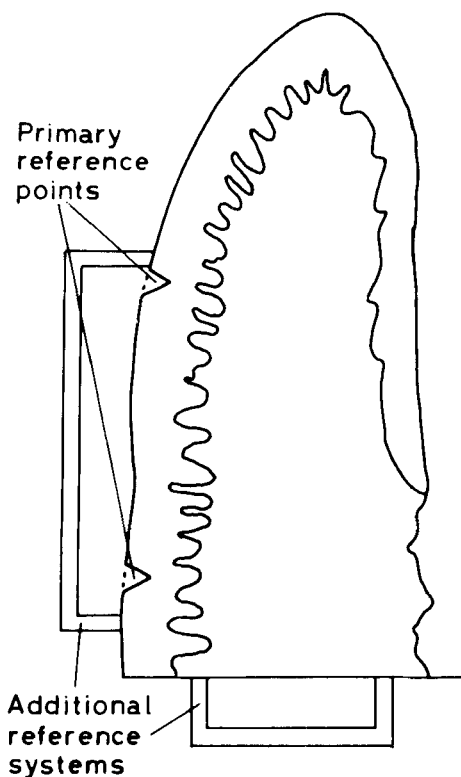


Fig. 6. Paper replica representing a histological section of gingiva. Additional reference systems formed like handles have been placed adjacent to both epithelium and connective tissue.

Whilst serving for accurate orientation of the serial paper replicas the reference points may sometimes break the integrity of the structure to be studied. In cases where the morphology of the tissue surface is simple, the broken outline produced by the reference points may be redrawn without producing major artifacts. It may often be convenient to eliminate the reference points in this way and replace them by an additional reference system produced outside the tissue. Similarly, additional reference systems must be produced when separate models of more structures within the same specimen are desired. Such reference systems, which may have the appearance of a handle (Fig. 6), are drawn on the first paper replica in a proper relationship to the tissue. In a negatoscope, the second replica is then superimposed on the first according to the primary reference points and by means of a cardboard or acrylic template, the handle is traced on to the second replica.

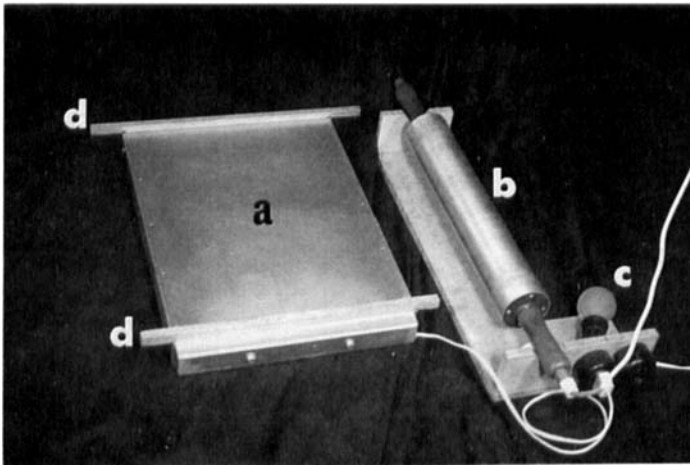


Fig. 7. The apparatus for wax plate casting consists of an electrically heated aluminium table (a) and a roller (b) which are serially connected. The table is shunted by an electrical bulb (c) placed on the roller support. Wooden lists (d) close the table at both ends.

This procedure is repeated for all replicas. In this way the additional reference system can be transferred to any number of replicas. Subsequent to the fusion of the wax plates (see later) these markers are removed.

The production of wax plates. The wax mixture for production of wax plates have the following composition.

Yellow bees' wax	5 parts
Paraffin wax	1 part
Crushed resin about 10 g per	1 kg

The ingredients are heated to approximately 110°C and melted together.

The apparatus for plate casting (Fig. 7) consists of an electrically heated, polished aluminium table and roller which are serially connected. The table is shunted by a resistance (electrical bulb) (Figs. 7 and 8). The roller's support is provided with a switch which automatically switches off the current to the table and connects the roller directly to the mains when the roller is removed from the support. In this way, it is possible to increase the temperature of the roller and decrease that of the casting table.

If the temperature of the table or roller is incorrect, then plate casting is difficult. It is, therefore, necessary to regulate the temperatures during the production of the wax sheets. The temperature of the table should be low (approx. 40°C) and that of the roller high (approx. 90°C).

The sides of the table which are adjustable, control the thickness of the wax plates. The technical design for the apparatus is shown in Fig. 8.

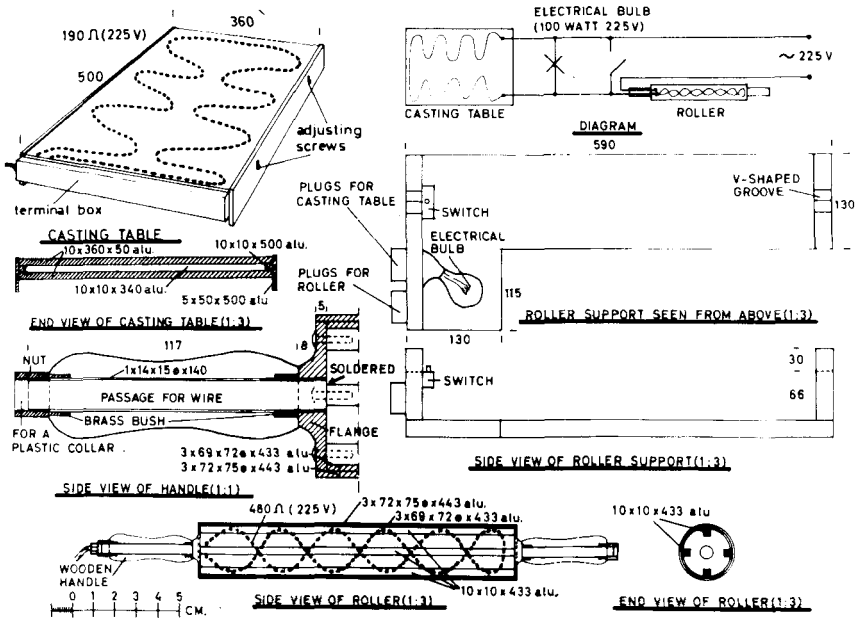


Fig. 8. Design of the apparatus for wax plate casting. The dimensions are indicated in accordance with the scale in the lower left corner.

Prior to the actual fabrication of a wax plate the table is covered by a sheet of tissue paper placed with its edge along one of the sides and moistened with turpentine. The tissue paper is further adapted to the surface by a brush and excess paper is cut along the opposite side. Wooden lists (Fig. 7) are placed at the ends of the casting table and melted wax poured evenly over the plate. As soon as a surface membrane has formed, the lists are removed and excess wax eliminated by rolling over the surface at an appropriate speed. After having been allowed to set for 2–3 minutes, the soft wax plate supported by the tissue paper cover is carefully removed from the casting table and allowed to set for a further 2–3 minutes on a plane surface. The wax plates, interleaved with paper, may be stacked and kept under pressure (approx. 15 kg) for 24 hours. The load is then removed and the plates left to dry for 2–3 days before use.

Production of wax models. The surface of the wax plate, not covered with tissue paper, is moistened with turpentine and a paper replica is placed upon it. Air bubbles between the replica and the plate are removed by the use of a rubber roller. The plate is allowed to dry for 2–3 days.

After the wax plates have been cut according to the outline of the paper replica, the plates are mounted on top of each other on a plane surface in

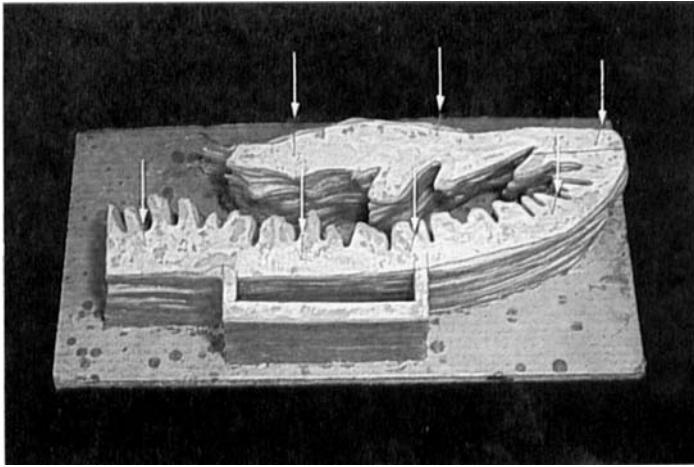


Fig. 9. Wax plates representing gingival epithelium are mounted on top of each other. The reference systems (handles) coincide exactly. Pins are inserted at different locations (arrows).

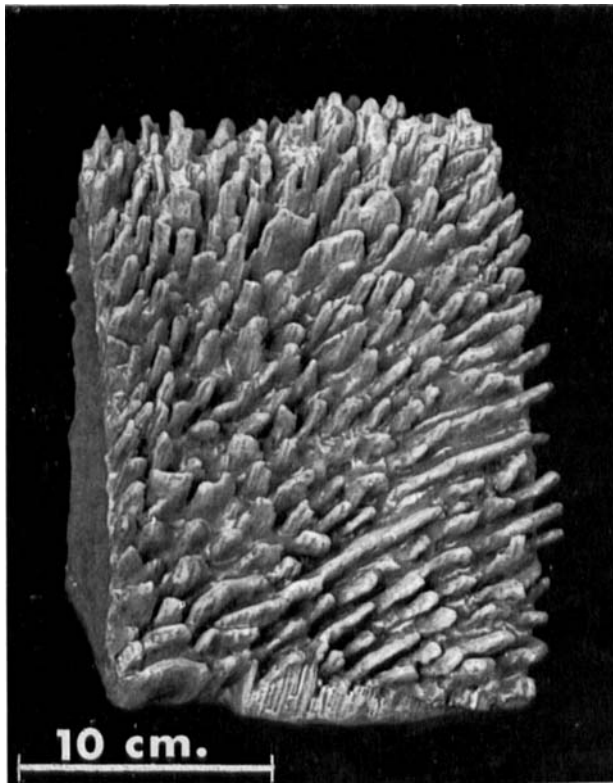


Fig. 10. A completed model ($200\times$) of the gingival connective tissue. The model is produced from 80 serial sections.

such a way that the reference systems coincide with each other exactly (Fig. 9). To prevent inversion of the model the plates have to be piled in reverse order of the section sequence. The plates are fused together at their edges by means of an electrical wax knife made from a soldering iron (30 Watt, 225 V). In order to obtain an appropriate working temperature, however, two of these have to be serially connected. Strengthening of the model is accomplished by inserting a few pins at different locations for every fourth or fifth plate (Fig. 9, arrows).

Although the model can be considered completed at this stage, a smoother surface is obtained by gentle use of the wax knife. The paper on both sides of each wax plate prevents the introduction of artifacts in the model.

DISCUSSION

The production of three dimensional wax models is a valuable method in micromorphologic studies of biological structures, provided the models accurately reproduce the original specimens. Although great care is exercised during the histological processing, some shrinkage of the tissue will occur during fixation and dehydration, and some distortion of the sections during cutting is bound to take place. Therefore, these factors must be taken into account during the evaluation of the final results. Variations in the thickness of the sections may also introduce an error into the three dimensional model. In cases where this error is supposed to influence essentially the results, the correspondence between the true thickness of the sections and the reading on the microtome scale should be tested (*Pusey, 1939*).

The fact that the greatest outline of the sections is always projected on to the paper replicas implies a slight enlargement of the final model. This is of little consequence at low magnifications (300–400x), but can be quite conspicuous at higher magnifications. The problem may be alleviated by focusing during projections and by cutting the edges of the wax plates accordingly (*Peter, 1942*).

In cases where the reference points cannot be made without interfering unacceptably with the structure to be reproduced, the specimen can be embedded together with a black painted (Indian ink-gelatine) sheet of liver tissue (400–500 μ) placed in close contact with the specimen. The liver tissue then serves as a bed for the reference points.

In contrast to previous methods (*Born, 1888; Peter, 1906; Peter, 1927*), the production of wax plates is based on a casting technique. This technique offers the possibility of producing plates of an accurate and reproducible thickness and has made the production of wax models less time consuming.

The time needed for the fabrication of three dimensional models obviously depends on the complexity and size of the specimen, but generally, the completion of a model of the marginal gingiva (1.5 mm) (Fig. 10) as based on 80 sections, will require approximately 8 hours.

Acknowledgements. The authors want to express their appreciation to Mr. Hans Thagaard Jensen for his excellent assistance during the design and production of the technical equipment.

SUMMARY

An improved technique for the production of magnified three-dimensional wax models of microscopic biological structures from histological sections is described in detail.

The improvements comprise the production of reference points of controlled depths in the tissue and the use of electrically heated equipment for the casting of wax plates. This makes the production of wax models less time consuming and may facilitate the study of three dimensional morphology of biological structures.

RÉSUMÉ

AMÉLIORATION DE LA TECHNIQUE DE PRODUCTION DE MODÈLES EN CIRE DES STRUCTURES BIOLOGIQUES

Les auteurs décrivent en détail une technique améliorée pour la production de modèles de cire à trois dimensions représentant à une grande échelle les structures biologiques microscopiques de coupes histologiques.

Ces améliorations comprennent l'établissement de points de référence de profondeurs contrôlées dans les tissus et l'utilisation d'une instrumentation à chauffage électrique pour la coulée des plaques de cire. Ainsi se trouve raccourcie la durée de la production des modèles, en même temps que l'étude de la morphologie tridimensionnelles des structures biologiques peut être facilitée.

ZUSAMMENFASSUNG

WEITERENTWICKLUNG DER METHODE ZUR HERSTELLUNG VON WACHSMODELLE BIOLOGISCHER STRUKTUREN

Eine verbesserte Technik für die Herstellung vergrößerter dreidimensionaler Wachsmodelle mikroskopischer Strukturen von histologischen Schnitten wird in Einzelheiten beschreiben. Die Verbesserungen umfassen die Her-

stellung von Referenzpunkten kontrollierter Tiefe im Gewebe und die Anwendung elektrischer Geräte für das Giessen der Wachsplatten. Dieses macht die Herstellung von Wachsmodellen weniger zeitraubend und erleichtert die Untersuchung der dreidimensionalen Morphologie biologischer Strukturen.

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