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A CONTRIBUTION TO THE TECHNIQUE OF PREPARING ANATOMICAL SPECIMENS OF BONES, ARTICULATIONS AND SMALL SKELETONS WITH INTACT JOINTS

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The bone, articulation and small skeleton preparations with intact joints are of essential importance for the training of medical students, for establishment of anatomical museums as well as for research work. Three basic stages are distinguished in the working technique of such preparations, namely: maceration of the soft tissues, defatting and bleaching. The stage of greatest importance is the maceration. Most of the methods of maceration are biologically conditioned, i. e. based on the natural property of the tissues to putrefy. In warm water the maceration occurs in one or two months. With skilfull regulation of temperature this process could be completed in 6—10 days (Mechanic — 1935, Yaroslavtzev — 1961). The speeding up of the process is obtained by means of adding Bact. proteus culture (Sorokin — 1942) or by pouring the material with uric acid (Bordas — 1928, Dyakonov — 1935). Chemical methods of maceration also exist, based on the property of certain chemical substances to destroy organic matter. With this purpose potassium hydroxide solution was employed (Petrov — 1896, Ivanov — 1946). Antiformin is also used, received by mixing chlorosis and sodium hydroxide during which reaction oxygen is freed, responsible for the destruction of organic substances. Andreev (1955) reports good results with the application of this method. Defatting of bones is obtained by various methods: boiling in sodium hydroxide solution, immersion in petrole, weak solution of sodium peroxide etc. For the bleaching of bones solutions are used calcium hydroxide, hydrogen peroxide, sodium peroxide and others.

The making of articulation and small skeleton preparations with intact joints is based on strictly specified maceration providing for the differential destruction of soft tissues and preservation of cartilages ligaments and membranes. For this aim solution of sodium hydroxide was successfully applied for the preparation of skeleton of small animals (Bulgakov — 1925). Andreev (1955) points out to the possibility of using antiformin maceration in preparing articular specimens. For making small skeletons of fetuses and infants, Yaroslavtzev (1961) applies biological maceration with cleansing in several stages and use of formalin for reenforcement of ligaments and preventing their putrefaction.

In spite of certain successes, the actual state of the problem under consideration is unsatisfactory in a number of aspects. The established

in practice biological maceration evolves very slowly and is provided with a number of shortcomings of hygienic nature. Maceration with chemical substances has undoubtedly advantages as far as speed and hygienic requirements are concerned. Yet, it is obviously disregarded, very probably due to the uncertainty of the preparation qualities. Moreover, at the present moment there is no adequate method for the preparing of fetal and small children skeletons with intact joints. The method described by Yaroslavtzev, based on the biological maceration, is rather complicated, non-hygienic and entailed with the use of formalin which accounts for the yellowish colouring of the specimens and thereby for their worse quality.

In the light of the unsettled state of the problem discussed, the elaboration of a new technique for making preparations of different bones, articulations and skeletons with intact joints was considered warranted; the new method would be in accordance with the requirements regarding the quality of preparation, speed of working process and safeness of working personnel.

In facing the task, we payed greatest attention to the method of maceration. Following a previous studying of existing maceration methods and carrying out of trial experimentation, we directed our attention to the chemical maceration. The latter meets the hygienic requirements and is produced relatively quickly. The basic item of experimentation appears to be the establishment of the conditions under which certain chemical substances could be applied for maceration in the preparing of various types specimens. Against the background of literature survey, it is clearly evident that chemical methods of maceration, here to fore, are applied in rather restricted scope, while for fetal and small children skeletons, as well as for cartilaginous-fibrous skeletons (for example larynx) they have not been used at all.

For maceration we used liquid containing 2% dry sodium hydroxide and 0.5% chlorosis lime[1]. The sodium hydroxide is solved separately in water, warmed up to 50°C, and the chlorosis lime with view to a better solubility — in lukewarm water, after which both solutions are mixed up. The products of the antiformin reaction act as maceration factors — mainly chlorine and oxygen — and along with them the free sodium hydroxide as well. Thus the maceration liquid applied by us was in fact antiformin-sodium hydroxide. The quality of the maceration with the composition of the solution just described depends on the previous preparation of the material and on the duration of the process at given speed of the latter, which is accordingly determined by the temperature of the maceration liquid. The soft tissues are cleansed prior to maceration, whereas fetal and children bones should not be fully denuded with view to avoiding their destruction by the solution. It is recommendable to keep the unfixed bones in water for 24 hours for washing them from the blood; bigger bones should be perforated with holes around the epiphyses for enabling the penetration of the liquid in depth. Maceration should not last until full detachment

[1] For preparing the maceration liquid Andreev employs "40% caustic soda in a quantity 2% of the solvent and chlorosis lime — 0,5% of the same quantity" (cited according to Andreev).

of the soft tissues occurs, for otherwise, in carrying out the subsequent procedures, the bone integrity could easily be damaged. Next washing with tap water is performed and removal of the soft tissue remnants as well. After drying of the bones we proceed with their defatting. The latter is carried out with petroleum for a duration of 2—4 days. Provided additional defatting is required, the bones could be immersed in aceton for further 24 hours. Under the action of the aceton the rest of the adipose tissues are extracted and a better white colour of the preparation is secured. Small size and finer objects could be defatted only in alcohol for 24 hours. After washing, the bones are bleached in hydrogen peroxide in 1:3 dilution for about 24 hours, and thereafter sun dried. During the bleaching process the cartilaginous remnants along the articular surfaces and periosteum should be removed.

We applied maceration for the treatment of bones not only of fresh, non-fixed cadaveric material, but also of fixed with carbol-formalin cadavers. In treatment of cadaveric skeletons fixed with carbol-formalin, the fixation does not exert appreciable effect on the speed of maceration. In these instances the bone specimens obtained have a yellowish shade, but nevertheless they are far more acceptable than those obtained with the common biological maceration. An essential condition for the good result is the assessment of the nature of the material liable to treatment. At equal concentration of the maceration liquid, its effect is determined by the initial temperature and by the duration of its action.

For the preparation of bone specimens from adults, at initial temperature of the liquid 50°C, the maceration process lasts from 4 to 5 days, whereas at initial temperature 80°C — from 2 to 3 days. Maceration of the spine and pelvic bones is the slowest. The higher the temperature, the quicker and more complete is the striping of soft tissues. Bone preparations could be made very rapidly provided the maceration liquid is warmed up to the point of boiling; with the latter method even the most difficultly susceptible to maceration bones get ready in 35 — 45 minutes. Apropos, it must be added that there is no particular hazard for damaging the preparations provided the course of maceration is carefully controlled.

In preparing bone specimens from fetal, newborn and children cadavers the maceration takes place in a markedly shorter term — it is carried out at initial temperature of the maceration liquid 50°C, immersion of the material for 30—48 hours, depending on the age and constant control of the maceration course. Without additional defatting, the preparations are kept in hydrogen peroxide for about 30 hours, followed by immersion in water for further 1—2 days, when the cartilaginous parts and the suturae bloat and are readily detached from each other.

For making articular preparations from adults at initial temperature of the maceration liquid 50°C, the immersion therein lasts about two days. The speeding up of the maceration by increasing the initial temperature of the liquid should be avoided on account of the risks of damaging ligaments and membranes.

According to the method described here, we succeeded in making larynx specimens, removed from non-fixed cadavers. The soft tissues are roughly cleansed and following dipping in the maceration liquid for about 4 hours, the final washing and cleansing is carried out. Thus the entire skeleton of the larynx is preserved together with conus elas-

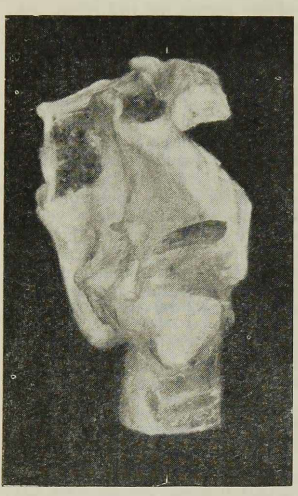

Fig. 1

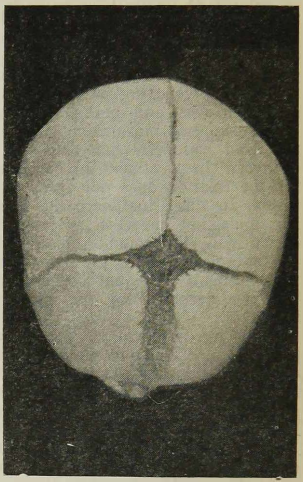

Fig. 2

ticus, membrana quadrangularis and membrana thyreohyoidea. Following bleaching with 15% hydrogen peroxide, the cavity of the larynx is packed with gauze with view to avoid deformations with drying. The preparation thus obtained gives a proper idea about the laryngeal skeleton, and serves as a valuable facility in training medical students. For preparing separate cartilages of the larynx, it is necessary to prolong maceration with further 1½ — 2 hours.

We also gained experience in preparing entire skeletons from human fetusses (from the fourth lunar month to newborns). We found out that in this instance the temperature of the maceration liquid assumes a particular importance. It is preferable to work with a liquid at room temperature, thereby changing only the duration of its action. In order not to damage the exposed parts with the maceration liquid, the previous cleansing of soft tissues should not be quite thorough. This applies particularly to younger fetuses in which the cartilagineous components of the skeleton are much more numerous. The soft tissues are not re-

moved from the fingers (in fetuses from the 4th to 7th lunar month), from the palms and soles (in fetuses from the 4th to 7th lunar month), from the ribs (fetuses from the 4th to 6th lunar month). The immersion in the maceration liquid lasts from 12 to 40 hours, depending on the age of the fetus, e.g. for fetuses in the 4th lunar month the duration is 12—15 hours, in the 6th lunar month — from 20 to 24 hours, in the 8th lunar month — from 26 to 30 hours and for newborns — from 36 to 40 hours.

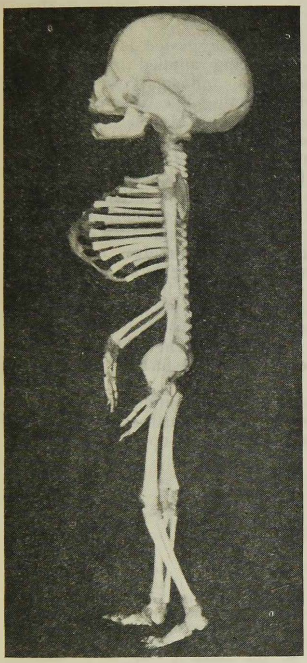

Fig. 3

In making skeleton parts with natural ligaments from 3-year-old children, the ligaments of the spine, pelvis, ribs etc. remain intact provided the already described method of treatment is used. In the preparations thus obtained the Y-shaped cartilage of the innominate bone is very well preserved , the ossification nuclei in the plantar bones are distinct etc.

After maceration the washing with tap water and defatting is carried out. The latter is performed with petroleum or alcohol for a duration of one day, whereas younger fetal skeletons needn't be defatted at all. Bleaching is secured following immersion in perhydrol, 1 : 3 water dilution, for 24 hours. After the bleaching the soft tissues, if such are found, are carefully trimmed with scissors. The skeleton is fixed on a stand in the position desired. In order to obviate deformation of the chest cage, it is packed up with gauze balls. Two or three days later the preparation is ready.

In preparing fetal skeletons we should always conform to the following conditions: the material liable to processing must not be previously fixed in formalin solution and the cadaveric decomposition must be forestalled for it would effect unfavourably the colouring of the preparation.

The characteristic features of the skeletons thus obtained are strength of bones, solid articular ligaments, transparent cartilages, intact fibrous and synovial membranes an agreeable white colour, light weight and

maintaining of the posture established during the drying process. The general configuration is preserved not only of the bones, but of the cartilaginous tissues as well. The articular capsules and ligamentous apparatus are comparatively well manifested for the age, the fontanelles being with preserved membranes. The skeletons constructed illustrate the dynamics of growth and shaping of the fetal skeleton, as well as the development of bone formation. The preparations remain unaltered with the course of time.

An essential advantage of the technique suggested is the fact that it provides for the chemical maceration being applied in making all sorts of preparations — bones, articulations, and small skeletons with intact jonts of all kind. Satisfactorily meeting the requirements for speediness and hygiene, the technique in question also secures a higher quality of the preparations. The bones are white, clean, neat, total and strong. As regards articular preparations, an outstanding achievement is the preservation not only of cartilage and ligaments, reported by other writers also, but of the membranes as well. The latter secures the preparation of specimens from the larynx with elastic membranes (fig. 1). from fetal and newborn skulls with clean, intact membranes of the fontanelles (Fig. 2) etc. The technique proposed by ourselves represents a substantial contribution to the problem of preparing fetal and newborn skeletons with intact joints (Fig. 3). As it has been fointed out they are of best quality. The working technique for similar preparations as described by Yaroslavtzev in 1961 is rather difficult, continuous, nonhygienic and, due to the using of formalin for packing the articulations, accounts for the yellowish colouring of the preparation, still further reducing its quality.

From the above it is clearly evident that the technique proposed responds satisfactorily to all the requirements and is further endowed with certain advantages in comparison to methods applied up to date.

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ВКЛАД К ТЕХНИКЕ ПРИГОТОВЛЕНИЯ ПРЕПАРАТОВ КОСТЕЙ, СУСТАБОВ И МАЛЕНЬКИХ СКЕЛЕТОВ С ЕСТЕСТВЕННЫМИ СОЕДИНЕНИЯМИ

В. Ванков, М. Христова

РЕЗЮМЕ

Сообщается техника приготовления препаратов костей, суставов, гортани, а также и скелетов плодов и маленьких детей. Для мацерации применяется раствор из хлорной извести и едкого натра. Приводятся данные о мацерации при производстве разных видов препаратов, а также и о следующих манипуляциях обезжиривания и выбеливания. Предложенная техника соответствует требованиям гигиенного характера, а также и таковым к скорости процессов и высокому качеству препаратов