PRESERVATION OF OSSEOUS AND HORNY TISSUES.

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INTRODUCTION.

During the winter of 1909–10 the large collection of hippopotamus skulls made by the Smithsonian African Expedition, under the direction of Col. Theodore Roosevelt, in British East Africa brought forcibly to notice the necessity of preventing the cracking, scaling, and rupture (at times explosive) of tusks, which within a month or two after their arrival often became badly disfigured.

Experiments for the accomplishment of the object soon showed the possibility of such a conservation. Acting upon the hypothesis that expansion and contraction are principally due to absorption and desiccation, a check to these, as complete as possible, seemed called for, and experiments were consequently conducted in accordance with such premises.

Under normal conditions during the life of the animal not even the enamel of the teeth shows signs of scaling. One and the same fluid permeates all their tissues, however different in density, chemical composition, and conductivity, forming one homogeneous unit, homogeneous to the active and normal forces of expansion and contraction.

To preserve natural objects successfully it is necessary to imitate the conservative forces of nature and meet their imperative demands. We must restore the homogeneity lost by desiccation, and to do so fully and permanently a medium must be found that will be sufficiently fluid to enter the most delicate capillaries and also sufficiently indestructible to resist normal chemical atmospheric changes, such as are likely to be found in a museum.

Various substances for this purpose have been considered. The retention of fats and of animal matter in skulls and bones, for example; the infiltration of oils, of glue, of shellac; but not one of these is satisfactory when one comes to consider its resistance to chemical changes or its ability to completely exclude the air.

Since it is the oxygen of the atmosphere and the accidental acids in the latter that attack the more complex molecules of matter, acting the more intensely the greater the humidity of the atmosphere, our aim, for the purpose of preservation, should be absolute exclusion of air and moisture. This atmospheric attack, combustion, or destructive metabolism, however it may be called, begins in organic life with the formation of the first cell and does not end until the last particle of bone has been disintegrated and oxidized into its component oxides. This is the course of nature, whatever the length of time, be it a few weeks or a million years. Silicification and carbonization as well as a complete replacement by calcium carbonate will, for the purpose of preservation, probably be always out of question. Infiltration of fluid calcium carbonate would be very slow and would probably change the external form while filling by accretion the cavities and capillaries, within and without, proving this method to be wholly unreliable.

There remains, therefore, as far as can be ascertained at the present time, only the one method, and that is the exclusion of air and humidity, and with it the forces of expansion and contraction. When life or vital forces no longer offset destructive metabolism, the latter will continue unchecked so long as external conditions promote the process. The same atmospheric air that consumes the worn-out tissues to furnish heat will continue to do so when heat is no longer necessary. As it is desirable to preserve museum specimens, our aim must be to check this combustion by the exclusion of the fuel necessary for its support.

That exclusion of air is an absolute preservative has been proven by the thousands of perfectly preserved insects and other animal inclusions in amber that have stood the test of thousands, if not of millions, of years. Of all the substances known and considered so far for the purpose of preserving bones and teeth not one is free from objections, not one even produces absolute homogeneity or absolutely excludes the air.

Among the expedients considered is the method of leaving within the bone a part of its organic and fatty substance as a preservative, a method that at first gives a better appearance as well as a substantiality to the delicate portions that are not evident when the full amount (50 per cent) of organic matter is withdrawn.

This might be satisfactory if it were possible to guard against changes of temperature and the entrance of air and moisture. This organic tissue within the pores of the bone, honeycombed (microscopically) by the taxidermic processes of treatment, offers ready admission to air and moisture, and as soaps or alkaline oxides, carbonates or borates, whatever may have been used, add nothing preservative in place of the substances removed, but rather assist in removing bone tissue, it becomes quite evident that a core or skeleton of such material can not be long-lived.

If it is undesirable to leave organic tissue within the bone unprotected, it is still more so if fats remain there. In cleaning the same the process is one of saponification. Now, rancidity is partial saponification, whereby strong-smelling fatty acids have been set free. These fatty acids are unsaturated molecules and at once attack the lime salts of the bone, forming lime soap, so often observed in the use of hard waters. The bone is in a state of dry rot. The retention, therefore, of fats in the bone during cleaning, or the infiltration of similar easily decomposing substances, instead of being an advantage would soon lead to disaster.

Another point to be considered is the following: The greater the amount of animal matter retained the less will be space for the preservative to penetrate, and the thinner, therefore, the crust of the latter, the sooner it will be broken by the gases of decomposition formed within and destroyed through the entering air and moisture.

White shellac has been recommended as another preventative. Shellac, to be sufficiently fluid, must be dissolved in at least 10 times its volume of alcohol. This solution does not undergo any chemical change and contains, therefore, one molecule of unchanged shellac to every 10 molecules of unchanged alcohol. If we now suppose that an object such as a tusk or a bone had absorbed a sufficient quantity and then had been removed from the bath, the drying or hardening would begin at once on the exterior. Of this outside or superficial layer nearly 91 per cent would escape as alcohol, while the remaining 9 per cent of shellac would be attracted and adhere to its like in the next molecular surface below. This escape of alcohol and adhesion of the shellac molecules would continue until a pellicle was formed, continuous around the object if the porosity were microscopic, or continuous with the sinuosities or depressions if more coarsely porous. This pellicle would not prevent the evaporation of the underlying mixture of alcohol and shellac; indeed, it hardly retards it. It is simply a covering of loosely cohering plates, irregular and greatly disruptured, with air spaces greater than the solid material, and necessarily so, because as the evaporation proceeds inward the outside material grows firmer and more unyielding, thus leaving a loosely spongy mass even under the most favorable conditions. The evaporating molecules of alcohol need and find a vent from the innermost capillaries through all the successive layers of shellac varnish. It is certain that the pellicle always gives passage to the solvent beneath and that in such a case as the one here mentioned the shellac remaining behind is only one-eleventh of the material absorbed by the object. It is, moreover, very probable that the shellac has too much of a colloidal nature to even reach the capillaries. What has been said of shellac applies equally well to all the colloids.

The same is true also of a varnish applied to a painting. It is a colloid substance inflated by a volatile liquid. The latter evaporates and leaves a thin porous crust of gum spread over the painting, uniting more or less with each superficial granule of pigment of the painting, which itself is a more or less porous layer on a ground composed of whiting and one of the ochers, united by a colloid. This in turn is necessarily porous, and is spread upon a very coarse, porous linen. Each layer penetrates and takes hold of the layer below. The porosity of such a varnish is proved by the dark tints of oxidation which it presents after the lapse of time and which are continuous throughout the thickness of the layer of varnish. It is further proven by the occasional oxidation of the oils used in the painting below the varnish, and the chemical change of the lead pigments into sulphides by the action of the sulphur dioxide contained in the contaminated atmosphere—all circumstances that make the restoration of a masterpiece a matter of great difficulty.

The use of gums and resins, or in fact any material that has a colloidal tendency, is wholly out of the question where absolute exclusion of air is demanded. Shellac is, however, a most excellent binder of decaying substances, and though the solution may not prevent air and moisture from penetrating, it is indispensable as a building or binding material.

The production of homogeneity would demand the infiltration of substances that would entirely fill up the interstitial spaces at ordinary temperatures and be as fluent as turpentine or benzine, so as to penetrate the most delicate capillaries. They should at the same time retain, if possible, some degree of firmness. To gain these qualities we are obliged to have recourse to various waxes, bitumens, or paraffins of the higher marsh-gas series. Stearin consists of stearic acid and glycerin and contains an unsaturated molecule that can not be depended upon.

Paraffin, as is well known, is fluid when heated, and will penetrate or dialyse, as it were, almost if not quite as readily as do lower members of the series, such as kerosene or benzine even when cold. The reason of this is that paraffin, according to Berlinerblau, contains as impurities products of the lower series, especially of a lower melting point, which, evaporating at or below 100° C., will work their way as vapor rapidly through the most delicate capillaries, then condense more or less upon the walls of the latter, and furnish points of attraction for products of a higher melting point, until finally the more slowly moving product of the highest point has penetrated the most deeply seated capillaries. The greater the volatility the greater also the diffusibility and power of penetration, and, on the contrary, the more a fluid approaches a colloidal condition the less it will penetrate.

Paraffin is not a perfect material for the preservation of objects, but it approaches perfection more nearly than any other substance known. It has the fault, however, of contracting upon cooling, and this contraction amounts to 14 per cent. Whenever an object has absorbed to saturation a fluid at 100° C., each capillary, at ordinary temperatures, will contain only 86 per cent of solid paraffin, crystallized, and 14 per cent interstitial spaces. These spaces may contain, besides the lower members of the series, also impurities, air, or even oxygen, according to Lippmann and Hawliczek, which has penetrated successively inward as contraction takes place, gradually following the cooling process. It is to be observed, however, that in a capillary or larger vessel the wall, rather than the intercellular lumen, attracts the layer. The slide test will prove this. Paraffin allowed to cool between a clean, dry slide and a cover glass will show a matted mass of fatty crystals arising from, or adhering to, a thin layer of the same on each glass.

Air, then, will filter through and approach the material which is to be protected, but does not reach it. These crystals act as a filter, like the cotton wad in a culture tube, only more thoroughly and on a more extended scale.

There is one danger, however, that is greater than the preceding. When by progressive inward cooling an inhalation of air takes place, impurities such as spores of fungi and bacteria may also penetrate one or two layers of crystals, and thus become embedded in the surface of the paraffin. These, if conditions are favorable, may attract moisture from the atmosphere, and according to Dr. O. Rahn,[1] grow and decompose the paraffin. If, as the above authority claims, paraffins of a lower melting point form better media for such growth than those of a higher melting point, the reason may be found in the more porous condition of the external layers owing to the greater evaporation of the lower series occurring as impurities in the grades of paraffins used. The growth of species of *Penicillium* and *Aspergillus* on the paraffin of fruit jars can be easily explained by the fact that the porosity of the crystallized paraffins, as well as the expension and contraction of the preserved material closely below the paraffin, even if the latter does not become detached from the sides of the jar by this movement, causes an alternate pressure of liquid outward or a suction of spore-laden air inward, and the conditions for a growth of fungus are perfect, provided the layer of paraffin is not too thick. If it be too thick no mold will form, but the air will enter and cause a more or less advanced state of vinous fermentation.

[1] Centralbl. Bakt., Abth. 2, Jena. 1906, vol. 16, p. 382.

An interesting experience of this kind was observed at the writer's home in some jars of preserved pineapple, which had been covered after cooling by a layer of paraffin three-eighths of an inch thick, and by a double sheet of commercial paraffin paper, tied securely over the same. After the lapse of three or four months a jar was opened and it was found to have changed into a well-flavored wine. The air had filtered through the three different layers and caused a slow fermentation, resulting in pressure sufficient to squeeze a part of the fermenting liquid through the layer of paraffin and evaporate the same to such a degree that the remainder stood nearly one inch below the layer of paraffin, which, with all this powerful pressure from below, had held its place in all the jars of preserves. Part of the juice, or wine, which had been pressed through still stood on the top of the paraffin, furnishing proof of that action.

Berlinerblau considers paraffin as "highly resistant to chemical influences" and that it can be attacked only by strong reagents at high temperatures. Therefore, in spite of its contraction, crystallization, and other imperfections, including the possibility of decomposition by molds or bacteria, and in spite of the darkening effect and the greasy touch, paraffin is at present the only substance that approaches in any considerable degree the conditions necessary for a perfect medium for the preservation of organic substances, such as teeth, bones, etc. Still this substance can be employed successfully only in case it has been infiltrated in its pure and uncombined state at a sufficiently high temperature.

Paraffin has been infiltrated into shells in a chloroform solution, but as this, according to Pawlowski and Filemonowicz, means only 2.42 parts of paraffin in 97.58 parts of chloroform, the amount that reaches the interior capillaries, even if the material (owing to its viscosity) should penetrate so far, which is more than doubtful, would be only 2.42 per cent paraffin and 97.58 per cent air space after the escape of the chloroform. The slight benefit derived from so small an amount infiltrated is, however, increased to some extent by rubbing the shells and crushing the crystals, thus forcing them into the pores, thus forming an impervious coating on the outside of the shell. Carbon bisulphide and ligroin give better results. They absorb, according to the above-named authors, respectively, 13.16, and 10.76 per cent. Such a polishing process (without chloroform) on a somewhat thicker layer of paraffin on fruit jars might also solve the problem of excluding the air in the case of preserving.

An advantage not to be underestimated is the prevention of animal depredations on the objects treated. Paraffin is not absolutely innocuous. Its toxicity, even laetal, has been proven in numerous cases, and if the poisonous quality is due to chance ingredients in paraffin, which can not be regarded as a chemical entity, the fact is not established. Kerosene, which differs from it only in the lower boiling point, is a recognized household insecticide. The fact that *Ascarides* and *Taenia serrata* are not affected by it does not disprove its poisonous qualities.

Experiments on herbarium specimens show an increase of flexibility, obtained by soaking in paraffin. The specimens are less brittle, will stand rougher handling, do not need to be poisoned and make a better appearance, although they lose their characteristic texture, which is often necessary in their determination.

WORKING METHODS.

The methods to be pursued in preserving osteological specimens necessarily vary according to their size, thickness, and purpose. If the object is merely to prevent cracking during prolonged overland transport, as in the case of tusks in Africa, it may suffice to carefully pour the melted paraffin of 80° to 90° C. into the open end of the tusk previously heated. In employing this process any sudden change of temperature should be avoided. After allowing the tusk to absorb the paraffin for a few minutes, the surplus material should be poured off. The outside of the tusk may then be treated with a benzine solution of paraffin, which should be well rubbed over every part of the surface, using as much as the same will absorb while exposed to the heat of the sun. If this has been well attended to, there is not much danger of longitudinal cracking, so undesirable in ivory intended for miniature painting, although it must be remembered at the same time the slow desiccation and gradual contraction are not checked altogether because of the tendency of the paraffin to liquify more or less in the heat of the sun during the day and to recrystallize as the temperature declines. This method will prove very satisfactory for tusks intended for industrial purposes, as it is undesirable to have the paraffin penetrate deeply.

If the purpose in view is permanent preservation, a large boiler of a sufficient size to allow the object to be immersed completely is heated in a water bath. In this boiler the object to be preserved is suspended by a wire from an hour or two for small skulls, such as those of rats and mice, to a week or more for tusks of a hippotamus. The heat in the experiments here described was turned on at 9 o'clock in the morning and turned off at half past 4 in the afternoon, and this alternation of heat and cold, in my opinion, proved beneficial, though at present I am not prepared to prove it.

To allow bubbles of air to escape from cul-de-sac hollows in the skulls, they were frequently shifted to different positions. When fully saturated, that is, when after long observation no more bubbles are found breaking above the object, it may be withdrawn from the paraffin and drained in various positions in the heat immediately above the surface of the paraffin. If too much paraffin has been removed, a rapid dip in the hot liquid, and subsequent draining in a hot place, or a rub, will quickly restore the deficiency.

Mr. G. S. Miller, in experimenting with weathered skulls, found that in five minutes—until bubbling ceased—the specimen had increased one-fifth in added substance and solidity to the fragile specimen.

NOTE.

Having after the lapse of 18 months reexamined 23 specimens treated in accordance with the foregoing method, I found:

First, that the tusks and some of the skulls were seriously disfigured by dust, but that a soft rag moistened with benzine restored the original color absolutely, a treatment that could only act beneficially to the specimens inasmuch as any chance outlet for decomposition gases is closed by this process.

Second, all the specimens examined were, without exception, intact. Not the faintest sign of disintegration was observable. This is nothing more than was to be expected. Paraffin from its very name, *parum affine*, denoting its chemical stability, neither attacks the calcium salts of the bone proper, nor does it permit its oxidation by the air, nor that of the animal matter still inclosed. Although the inevitable disorder connected with the transfer of specimens from one building to another subjected them to more than ordinary vicissitudes, there was, aside from dust, no damage apparent.

An interesting specimen well depicting the difference between a skull treated with paraffin and a jaw in its natural state is that of a leopard (Cat. No. 162927, U.S.N.M.), which was found on the ground in a considerably weathered condition. Here the jaw, with its countless cracks and crevices, worn more or less deep, with the bone surface here and there chipped off, exposing more and more the looser stratum within, and particularly the ragged, broken teeth, offers striking testimony in favor of the treatment.

The success being complete, it is not probable that an increase of the number of test specimens would have materially modified the results, especially as the experiment covered practically the whole ground of variation in size, condition, and age.

Another fact deserving attention is the displacement of animal matter and fats by paraffin. This vitiates the material infiltrated and impairs its stability. If rhigoline, petroleum ether, or benzine were used as a preliminary detergent, this waste of material would be obviated, since these can easily be distilled off from their impurities and used over again.

BIBLIOGRAPHY.

BERLINERBLAU, JOSEPH. Das Erdwachs, Ozokerit and Ceresin. Braunschweig, 1897, pp. 239. Handbuch der chemischen Technologie, Vol. 1, Gruppe 2, Abth. 2, Teil 3.

BERTRELLI, E. and MAZZA, C. Sopra un nuovo metodo recentemente proposto per rendere impermeabili le stoffe e gli abiti, trattandoli con paraffina. Riv. d'ig. e san. pubb., Torino, 1901, pp. 426, 472.

CATHOIRE, ———. L'impermeabilisation hydrofuge des vêtements par la paraffine. Rev. d'hyg., Paris, 1900, vol. 22, pp. 296–313.

CHABRIE, CAMILLE. Traite de Chimie appliquée. Paris, 1905–8.

DEBONO, F. Études des paraffines au point de vue pharmaceutique. Montpellier, 1886, quarto.

FIELD, J. C. and J. Paraffin, its origin, manufacture, and application. London, J. M. Johnson and Son, 1862, 31 pages.

GREGORIUS, RUD. Mineral waxes, their preparation and uses. Translated from the German by Ch. Salter. London, Scott, Greenwood and Son, 1908, vol. 7, pp. 240, 32 illustrations.

Калантаръ, А. Микроскоп. изслѣдованiе Бакунск. нефти и продуктовъ ея. (Microscopical investigation of the Baku naphtha and its products.) Тр. Бакун. Отд. И. Р. Текн. Общ., pp. 2–9; 107–114.

RAHN, O. Ein Paraffin zersetzender Schimmelpilz., Centralbl. für Bakteriol. (etc.). Abth. 2. Jena, 1906, vol. 16, pp. 382–384.

Ракузинъ, М. Оптическiй феноменъ Тиндаля и его значенiе для микроскопiи и геологiи нефти. (Tyndall's optical phenomenon and its importance in the microscopy and geology of naphtha.) Журн. Р. Физ. Хим. Общ. XXXVIII. vol. 4, p. 677; vol. 5, pp. 790–798.

———. Оптическое изслѣдованiе нефтей ферганскихъ со святого острова и Берекеиской. (Optical investigation of the naphtha of Ferghana, Holy Island, and Bereczk.) Журн. Р. Физ. Химическ. Общ. XXXVIII. 8. проток. pp. 1183–1184

RAKUSIN, M. Die Untersuchung des Erdöls und seiner Produkte. Eine Anleitung zur Expertise des Erdöles, seiner Produkte und der Erdölbehälter. Mit 59 in den Text eingedruckten Abbildungen. Braunschweig, 1906, octavo, pp. XVIII+271.

STRAUME, O. Paraffinum liquidum u. Vaselin. Inaug.-Diss. Jurjew, 1894, pp. 86. Contains an excellent medical and pharmacological bibliography.

WALTER, V. Beiträge zur Kenntnis der höheren Paraffine. Stuttgart, 1886, octavo.

Technical literature on paraffin is rare, except where it deals with its sources, and here the Russian literature under нефти (naphtha) and асфалтъ (asphaltum) is particularly plentiful. Almost as great is the medical literature under paraffin injection, paraffin embolism, and the toxicology of paraffin.