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Waldemar PILARSKI, Stanisław SERWATKA,
Krzysztof ŚWIEŻYŃSKI & Mieczysław WĘGRZYN

New Attempts at Fixing Anatomical Material of Large Mammals

Bisoniana XXVII

[With 9 Figs.]

Use of an appropriately adapted garden spray to inject fixative fluid, the composition of which is given in the study, permits of eliminating difficulties encountered in fixing the carcasses of large mammals. Fixed whole carcasses can be used for macroscopic investigations for a long period. The position, shape and colour of internal organs changes very little, which facilitates preparation and permits of obtaining good photographs. Histological preparations made from sections taken several months after the animal's death stain in a typical way and make it possible to trace the structure or organs.

The fixation of material intended for anatomical research is a very old problem. Studies in this field have continued to appear, their chief aim being improvement of methods, both from the aspect of their advantageousness and economy in use (Blum, 1893; Blum, 1894; Paulli, 1909; Możejko, 1910; Lubicz-Niezabtowski, 1924; Kubik, 1957; Franzke-Heinze, 1961, Schmidt, 1910; Erskine, 1961; Świeżyński, 1962).

A method of fixing the carcasses of large mammals (e.g. European bison) by means of injection into the arteries and storing the whole fixed carcasses has been applied in the Dept. of Animal Anatomy of the Veterinary Faculty of Warsaw Agricultural University. The data presented here form the result of five years of experiments in this field.

The carcasses of domestic animals — 1 dog and 7 horses — were first used as material for fixing by the method described. The carcasses of 12 European bison (which included 5 dead from natural causes and 7 killed and drained of blood) were next fixed. As the fixative fluid had to overcome considerable resistance in penetrating to organ and tissues it was necessary to apply pressure which would not at the same time

destroy tissue structure. It was decided to use a garden spray produced by the Wrocław Agricultural Engineering Works, type Op. — 20 (Fig. 1). Adaptation of the spray consisted in removing the rose and securing a double-nozzled connection of the gas type on to the end of the rubber outlet pipe (Fig. 1 — 1). Two plastic tubes 8.3 mm in diameter were then fixed on the nozzles, the tubes ending in metal cannules 3.7 mm in diameter for use on large animals (horses, bison). The free end of each cannule had three circular grooves enabling the joint to be firmly bound after the cannule had been introduced into the lumen of the blood vessel.

Various aspects were taken into consideration in searching for fixing agents, such as their cost and at the same their suitable quality and effect on the material fixed, bearing in mind that the latter would have to be used for both macroscopic and microscopic examination. The requirements made of fixing fluids are in principle satisfied by the mixture successfully used by Professor R. G e t t y, Department of Veterinary Anatomy, Iowa State University, Ames (USA).

Isopropyl alcohol	60%
Phenol	6%
Formalin	4%
Corn syrup or glycerine	5%
Water	25%

It was taken that the basic component of the fluid we used should retain the proportions of the corresponding substances and therefore only a qualitative modification of its composition was made, taking into account prices and availability of the substances in Poland. Isopropyl alcohol was replaced by methylated alcohol, which can be obtained without difficulty, and maize corn by potato syrup or occasionally glycerine. The fixing as modified by us therefore contained the following components:

Methylated alcohol	60%
Phenol	6%
Formalin	4%
Potato syrup or glycerine	5%
Water	25%

The ingredients were mixed in the order given above. The order of mixing is important when using syrup, as it prevents the particles of starch coagulating and settling in the form of sediment. Potato syrup used in the food industry was prepared by diluting it, using 1 part of syrup to 1 part of water, at a temperature of approximately 60°C.

The animals intended for anatomical research were anaesthetised with chloral hydrate administered orally, intraperitoneally or *per rectum*. The narcotic was combined with a dose of tranquilliser. After deep narcosis had been achived the animals were bled by introducing glass cannules to *aa. carotides communes* at the level of the lower one-third of the neck. After bleeding the glass cannules were removed and the metal cannules of the injecting apparatus were inserted. Injection was carried out while maintaining pressure of not more than one atmosphere in the tank of the apparatus. When fluid began to leak from the natural orifices of the carcass, such as the anterior nostrils, mouth or vulva, this was taken as a sign that a sufficient amount of the fixative liquid had

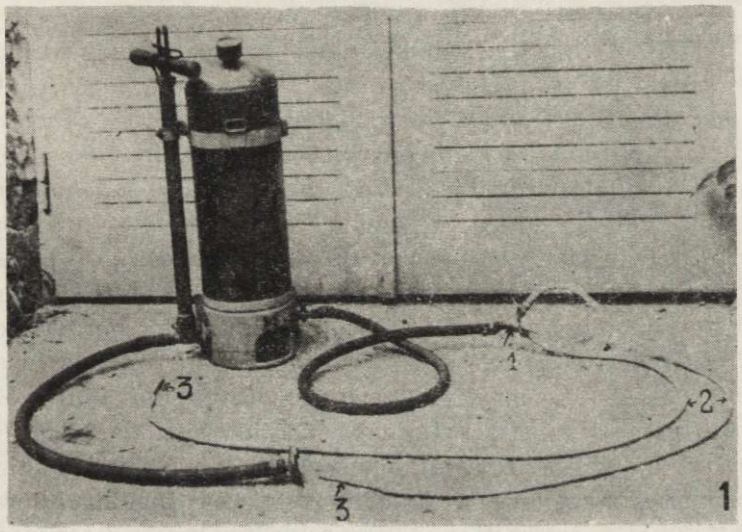

Fig. 1. Apparatus for injecting the fixative fluid.
1 — double nozzled connection, 2 — attached pipes, 3 — cannules.

been introduced. Some indication that correct injection had been effected was also provided by the hardness of the eyelids and stiffening of the conchae and tail.

The injected carcass was kept, on account of the lack of any other suitable place for storage (e.g. refrigerator) in the dissection room, in a recumbent position. When it was necessary to keep the carcasses for a longer period the circulatory parts of the limbs were protected from excessive drying by drawing sleeves of plastic material over them. During macroscopic preparation of the carcasses of 2 bison sections were taken from several of the organs for histological preparations. The sections were taken from carcasses which after fixing had been stored whole for a period of several months during the summer.

CONCLUSIONS

The method described has many advantages, enabling it to be used both for fixing scientific material and preserving carcasses intended for didactic purposes. The fixing process is relatively simple: the apparatus for introducing fluids is easy to obtain and not difficult to adapt. The adapted spray has yet another good point, i.e. it weighs only 14.5 kg. The fixative fluids used in our Department satisfied many of the requirements made of substances of this kind. The fixed carcasses can be used for both macroscopic and microscopic investigations.

a. Application to Macroscopic Examinations

The method of injecting whole animal carcasses enables material to be preserved in the form closest to the natural one. The skin integument is damaged to a very slight degree (only where the cannules of the apparatus are introduced). This ensures, especially in the case of species and also individuals with thick skins, that the tissues remain moist for a long time and dry up very slowly. The location of internal organs, when care is taken both before and during the injecting process, is very little changed and even during the operation of introduction of the fixative, part of the displaced viscera return to their normal position as the result of increased pressure. The shape of the organs, especially of parenchymatous organs, is maintained within the limits of natural deviations. After fixing depressions and impressions of all kinds are clearly visible, which frequently disappear when dissecting carcasses not previously fixed. Organs fixed in this way, making allowance for a slight degree of contraction, are suitable for many measurements for comparative purposes.

The use of fixative fluids containing alcohol and glycerine causes the contrasting colour of macroscopic preparations to be retained, which both facilitates the work of preparation and enables good photographs to be taken. Preparation of the carcass should, however, be started after a certain interval (in the case of large animals about 14 days, and with small animals appropriately earlier), to enable the tissues to absorb and »bind« the fixative.

Whole carcasses may be stored for long periods (the authors prepared material from a carcass fixed 1.5 years previously). After their dismemberment the various macroscopic preparations are equally resistant to drying and free from the action of mould. They may be stored in polyethylene bags. As the carcasses of experimental animals are drained of blood before fixing the arterial vessels are empty and it is therefore

comparatively easy to fill them with plastic mass for the purpose of making casts. After the soft tissues have been prepared the skeleton is available for use. Tests made in our Department prove that the fixative fluid inhibits the process of decomposition on only to a slight degree when material is macerated, while this process is rendered impossible when undiluted formalin is used.

b. Application to Microscopic Examinations

Sections intended for histological preparation proved to be well fixed. This method enables the fixative fluid to be introduced into almost living tissues. If the sections of tissue were obtained by means of operation on an anaesthetized animal the method, from the point of view of the interests of macroanatomy, would be less advantageous, since certain regions of the body would be irrevocably damaged during operation. When the method of injection of the whole carcass is applied, the order in which use is made of the various parts is of no importance from the histological point of view, as all the tissues are fixed.

Histological preparations were made in the Department from the liver, pancreas, parotid salivary gland, kidneys, spleen, adrenal glands, thymus and cerebral cortex, all of which were of full histological value. They did not exhibit any structural damage despite the fact that the samples were taken several months after the animal's death. They stain in a typical way and permit of the tissue structure being accurately traced (Photos 2—9). They are in no way inferior to preparations made by traditional methods (Zarzycki, 1956) and are decidedly better than preparations made from material kept in a water solution of formalin (Wilkus, 1957).

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Warsaw Agricultural University,
Department of Animal Anatomy,
Warszawa, Grochowska 272.

Waldemar PILARSKI, Stanisław SERWATKA, Krzysztof ŚWIEŻYŃSKI i Mieczysław WĘGRZYN

NOWE PRÓBY UTRWALANIA MATERIAŁU ANATOMICZNEGO DUŻYCH SSAKÓW

Streszczenie

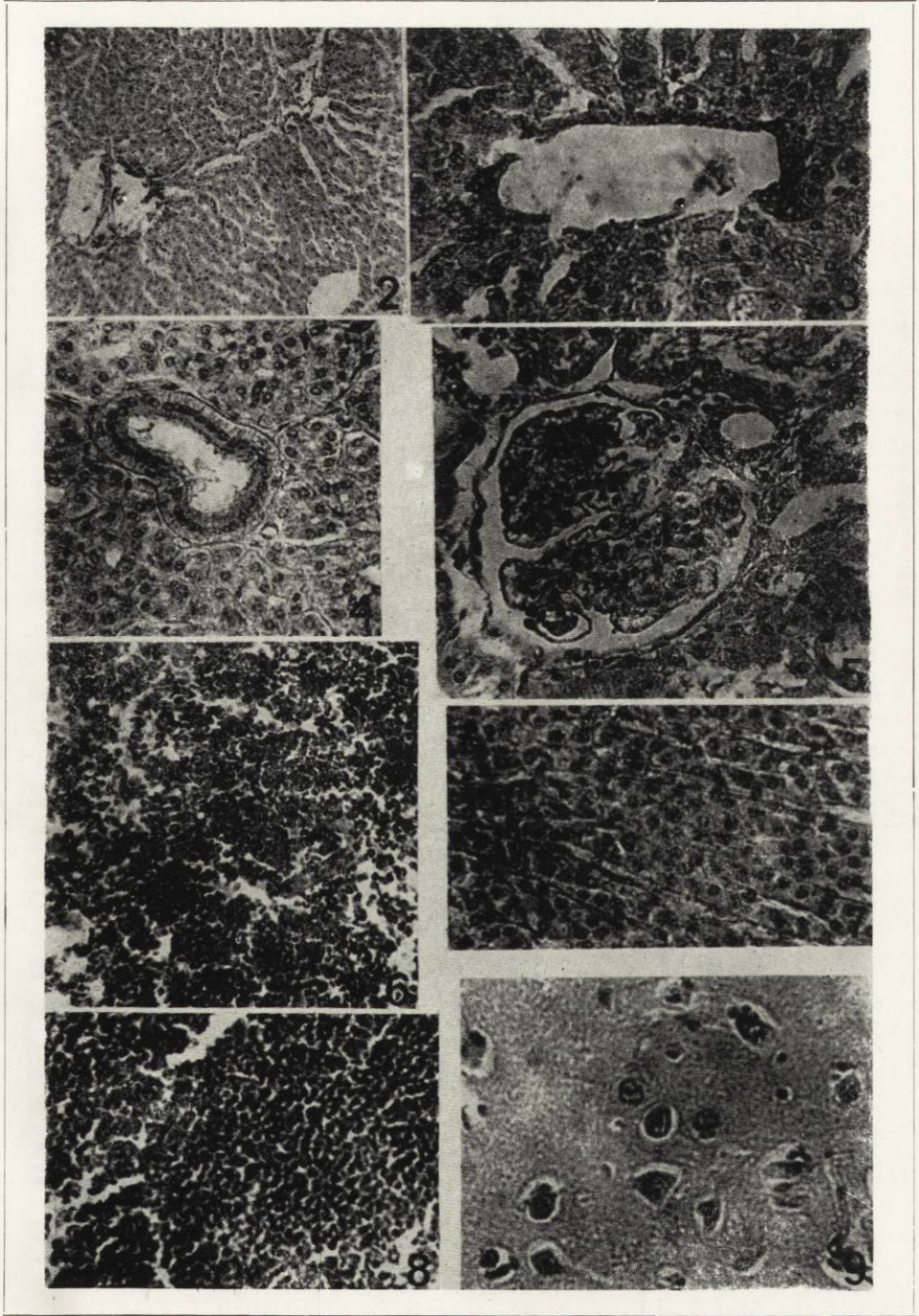
Użycie odpowiednio przystosowanego opryskiwacza ogrodniczego (Ryc. 1) i wstrzykiwanie przy jego pomocy płynu konserwującego o podanym w pracy składzie pozwala na wyeliminowanie trudności spotykanych przy utrwalaniu zwłok dużych ssaków. Utrwalone w całości zwłoki użyte mogą być do badań makroskopowych przez długi okres czasu. Układ narządów wewnętrznych, kształt i barwa są mało zmienione, co ułatwia preparację i pozwala na dobrą dokumentację fotograficzną.

Preparaty histologiczne, wykonane z wycinków pobranych w kilka miesięcy po śmierci zwierzcia, barwią się typowo i pozwalają na prześledzenie budowy narządów (Fot. 2—9).

EXPLANATION OF PLATE XXI.

Figs. 2—9. Microscopic structure of some internal organs of European bison, *Bison bonasus* (Linnaeus), fixed by described method.

Fig. 2. *Hepar*, 36 ×, Fig. 3. *Hepar*, 144 ×, Fig. 4. *Gl. parotis*, 144 ×, Fig. 5. *Ren*, 144 ×, Fig. 6. *Lien*, 144 ×, Fig. 7. *Gl. suprarenalis* (*zona fasciculata*), 144 ×, Fig. 8. *Thymus*, 144 ×, Fig. 9. *Cortex cerebri*, 144 ×.



W. Pilarski *et al.* *auctores phot.*