AMERICAN MUSEUM NOVITATES

Number 233 Published by THE AMERICAN MUSEUM OF NATURAL HISTORY New York City Nov. 12, 1926

59.07

MOUNTING BY PARAFFIN INFILTRATION

A METHOD FOR THE PERMANENT PRESERVATION OF WHOLE SPECIMENS OR DISSECTIONS

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There are certain groups of natural history and anatomical objects which, until the present time, have proved extremely difficult, if not impossible, to mount successfully. Any vertebrate devoid of a protecting cover of fur or feathers is not easy to mount in such a way that it looks alive. In the American Museum, most of the amphibians and reptiles have been cast in wax and accurately colored by skilled artists. In spite of the realistic appearance of the resulting object, especially when successfully posed in a habitat group, the casting method has certain drawbacks, all too obvious to the custodian of the exhibit. In the first place, the visitor often asks: "Is that the real thing, or wax?" Usually he immediately loses interest in the exhibit when he learns that the animals are "mere reproductions." Secondly, wax casts are very breakable, especially the delicate toes or other appendages. Lastly, a wax cast "to look alive" is a very expensive proposition, and only certain highly trained artists can produce acceptable work.

Not all museums, however, make casts of their amphibians and reptiles. Large specimens of the latter may be skinned and the prepared hide fitted over either a cast or a modeled manikin. This is a useful method for large, rough-scaled species, but softer bodied forms, especially those with fleshy scales, cannot be prepared in this way, for the scales either coil or shrivel. Snakes have always proved to be among the most difficult of vertebrates to mount successfully.

While museum curators have been struggling in this and other countries to make amphibians, reptiles, and creatures with similar skins look alive, there has been exhibited for a number of years in the Anatomical Institute of the University of Vienna a series of anatomical preparations which have lost little of their "fleshiness." Dr. F. Hochstetter, who is responsible for these preparations, has also mounted a number of reptiles, amphibians, and even mammals by the same method.

The latter series, although not on public exhibition, are well known to the herpetologists of Vienna, and possibly of other cities, for they are some of the finest preparations which have ever been produced.

It was clear from an examination of these specimens that they had been infiltrated with paraffin or wax. The exact details of the method, however, are not available for steps have already been taken to put the process on a commercial basis. It is to be hoped that before long mu-

Fig. 1. American toad. Mounted by the infiltration method as described.

seums will find in Vienna a source of supply for such beautiful exhibits as Professor Hochstetter has produced.

The general method of paraffin infiltration is, of course, well known to every histologist. It has been used in the laboratories of the American Museum as well as in those of other institutions for many years. This method has also been used in the preparation of whole specimens, such as brains. As the Hochstetter method is not available to us we have attempted to explore the possibilities of utilizing well-known histological techniques to obtain the same result. After a long series of experiments we have succeeded in producing mounted specimens which look, we

believe, more like the living animal than either the most successful casts or mounted skins. These specimens have retained their original form and most of the original colors. Only the eyes have proved difficult to infiltrate successfully. These, in amphibians, were replaced by colored pin heads while those of reptiles were later touched up with a little color. Interference colors, such as greens, or soluble colors, such as the oils in certain amphibians, are altered by the technique. Most colors, however, especially yellows, browns, reds, and even some blues, are unchanged by the process. The resulting specimens are more than the "real thing." They include besides the exterior, the interior,—the heart, liver, lungs, etc., in place and merely infiltrated with paraffin. As each cell in the animal's body is actually embedded in paraffin, there can be no doubt as to the permanent character of the preparation.

The technique finally adopted seems to be most suitable for amphibians and reptiles. No doubt, some modification would have to be made for infiltrating other types of preparations. We have, therefore, mentioned below the reagents which yielded a partial success, as probably some of these would be serviceable for other tissues. Practically all of the chemicals employed are available in any histological laboratory. The method should, therefore, be of especial value to university museums already supplied with histological laboratories.

For the best results, live animals, preferably those which have recently been caught, are desirable. However, successful results were obtained with animals fixed in formol or alcohol.

With the exception of large snakes, all live animals are anesthetized and killed in a solution of chloretone in water. Chloretone is preferable to ether or chloroform as it leaves the specimen perfectly limp and flexible so that it may be posed in any position. Ether in moderate doses is used for the larger snakes.

After the animal is dead, a 10% solution of formol is injected into the body cavity. Snakes are generally the exception to this rule, as the injected fluid distorts the body and makes it difficult to pose naturally. Rustless insect pins and a dish containing a half inch of hardened paraffin are useful in posing. After the desired posture is obtained, the animals are covered with fixing fluid.

A long series of experiments was necessary before the proper fixing fluid, which gave a minimum shrinkage and decoloration, was discovered. Various strengths of formol, formol plus acetic acid, formol plus sodium carbonate, and formol with calcium chloride were all tried and found unsatisfactory. While formol is an excellent fixing agent to

prevent shrinkage, nevertheless, it destroys the color. To counteract this, a weak solution of sodium carbonate was used which successfully retained the color while the animal was in formol but was ineffective when alcohol was used for dehydration. While formol acetic prevented shrinkage, the acetic acid increased the bleaching properties of the formol. The calcium chloride mixture caused too great a shrinkage. Oil St.

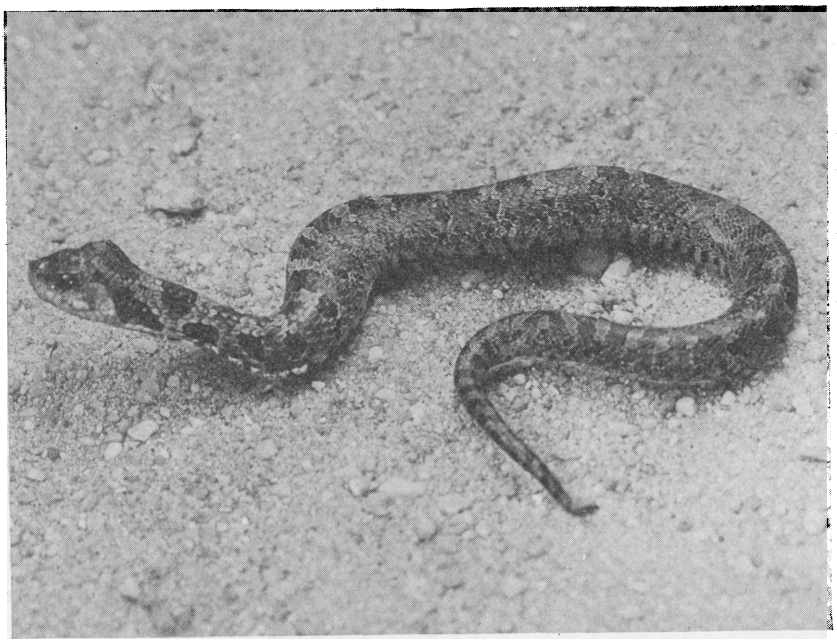

Fig. 2. Hog-nosed snake "spreading." Mounted by the infiltration method but absolute alcohol utilized instead of terpineol.

Rocco, which is a hardening, dehydrating, and clearing agent, caused the animal to shrink and darken. Carnoy's fluid decolorized.

Bouin's Acetic-Formol-Picric (full strength) was used and found to be the most satisfactory fixing agent, as no shrinkage occurred, and the pigments did not fade. The only drawback was a slight yellow tinge due to the picric acid. To counteract this, one part of full strength Bouin to one part of 10% formol was used with successful results. When the yellow color remained too predominant, the animal was washed in 10% formol until free from the excess picric acid.

Gradually increasing strengths of ethyl alcohol are used to dehydrate and harden. As alcohol has a tendency to take out the color, it is advisable not to leave the animal in it any longer than necessary. When in 50 or 70% alcohol the eyes are removed and glass eyes inserted.

Some difficulty was encountered in finding a satisfactory substitute for absolute alcohol or a clearing agent which would clear from 95% without shrinkage or darkening. Theoretically, cedar oil should have answered the purpose, but no cedar oil could be obtained which cleared perfectly from 95%. While anilin oil clears successfully from 70%, it permanently darkens the object. Oil of thyme clears 95% alcohol specimens, but also darkens them somewhat. Carbolic acid was unsuccessful. Clove oil cleared from 95% but also decolorized and caused considerable shrinkage.

The most satisfactory substitute for absolute alcohol was found to be terpineol. It clears from 90%, does not extract the color nor cause shrinkage. Care must be taken to wash out thoroughly the terpineol with several changes of xylol, as terpineol does not mix with paraffin. A mixture of equal portions of xylol and paraffin is used for infiltrations, followed by pure paraffin of 56° melting point. It is desirable not to make the paraffin bath any longer than necessary, as the constant heat darkens the specimen and makes it brittle. After the paraffin bath is completed, the animal is removed and melted paraffin injected with a warm hypodermic needle into the body cavity to fill the hollow spaces. By successfully manipulating the needle considerable "character" can be given to the specimen. Certain parts may be more distended than others. Again, the warm wax may be molded to a certain extent and various muscles brought into greater display. When the animal has been thoroughly injected it is immersed in ice water. Cooling in the air is successful for some specimens but darkens others. After hardening, the excess paraffin is removed with xylol, either on a stiff or a soft brush.

Specimens which have been preserved in formol may be embedded as above but, as their colors are usually altered, a subsequent coloring is necessary. Such an artificially colored specimen is shown in Fig. 3. In certain amphibians, such as the red eft, the colors are modified by the higher alcohols, and a tinted paraffin may be used to replace the color destroyed. Snakes and large lizards should be opened along the ventral surface as the penetration of the solutions through the integument is very slow. In short, a certain amount of ingenuity is necessary to obtain perfect results with all specimens. The great majority, however, will

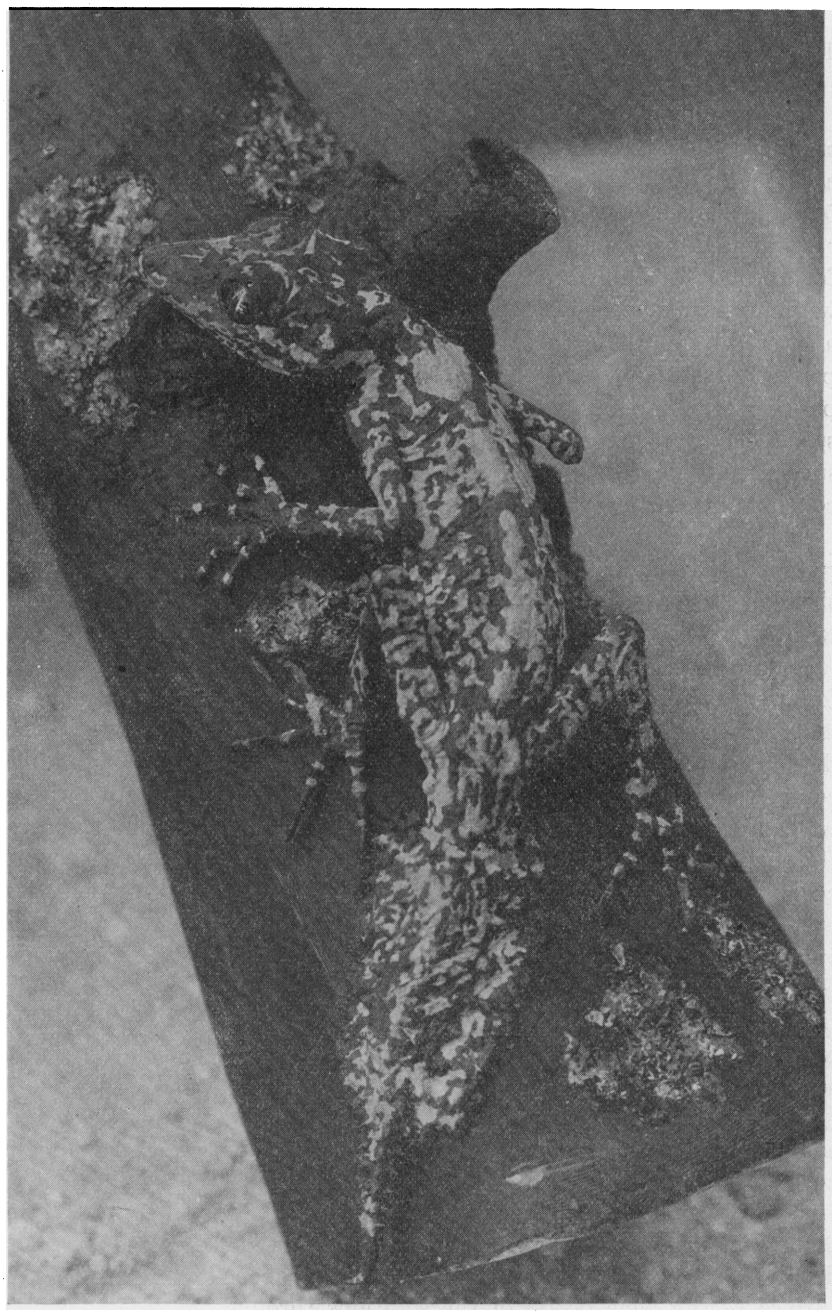

Fig. 3. Australian gecko (*Gymnodactylus platurus*) fixed in formalin in the field and preserved in alcohol. Infiltrated with paraffin. Colors restored with oils

lose little of their original color or form if treated as indicated above. We may briefly restate the method which we have found to be most serviceable for general work.

1. Chloretonize or etherize and pose the specimen;
2. Fix with Bouin's Acetic-Formol-Picric and a 10% solution of formol in equal parts;
3. Wash out excess picric with 10% formol and the weaker alcohols;
4. Run up gradually to 95% alcohol;
5. Transfer gradually into terpineol,
6. Wash with several changes of xylol;
7. Embed in 56° paraffin;
8. Inject specimen with paraffin and mold into life-like form;
9. Plunge into cold water;
10. Remove excess paraffin with xylol.

The time required for each operation will vary with the size of the specimen. It averages about a day for each solution.