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The Uses of Formaldehyde in Animal Morphology

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THE USES OF FORMALDEHYDE IN ANIMAL MORPHOLOGY.

BY GILBERT L. HOUSER.

By the term *formaldehyde*, I wish to designate a 40 per cent solution of the gas formaldehyde in water. Several articles answering to this description have been placed on the market under trade names such as the "Formalin" of Schering, the "Formol" of Merck, and the "Formalose" of Richards & Co. So far as I have tested these various preparations, they all agree as to composition, and yield perfectly similar results. My attention was first directed to formaldehyde as a morphological reagent in July, 1894, and I have been using it in my work, and have experimented with it in various directions since that time. It certainly possesses several most remarkable properties; so remarkable, in fact, that certain phases of laboratory work in animal morphology are ultimately destined to undergo a revolution through its use.

I. FORMALDEHYDE AS A GENERAL PRESERVATIVE.

It has been urged many times that the zoological specimens placed in the hands of students for class-work are too often mere caricatures of the living animals themselves, and that various erroneous conceptions about nature are thus sure to arise. Granting that we should, as far as possible, use fresh material for study, the fact remains that there are many animals which must be preserved if we are to study them at all. The whole of the group Echinoderma, and, with one exception, all the members of the Coelentera, are cases in point. Such animals have to be preserved at some distant point and transported to us. Now, formaldehyde has its most important and its most far reaching application in this particular field of morphological work. It is the best general preservative of material for class-work that has yet been discovered. The peculiar qualities which confer upon it this distinction are as follows:

First.—It does not extract water from the tissues and consequently it does not shrink them. The distortion of an animal will be in direct proportion to the shrinkage of its tissues, and this, in turn to the amount of water extracted. Hence it was that our attempts to preserve such watery forms as medusæ, ctenophores, etc., with our old media were always failures; our preserving fluids dehydrated them. Formaldehyde, however, will preserve almost every form of animal life known without any distortion. Such a fact opens up possibilities for class instruction which are almost ideal.

Second.—Most of the pigments of the animal body are not extracted by formaldehyde. This quality ranks next in importance to the preceding one. Natural coloration enters so largely into our conceptions of animals that bleaching during the process of preservation is always to be deplored. With alcohol as the preserving fluid, all parts are certain to be brought to the same level of dingy yellow after a time. But with formaldehyde, we can hope to show our students the colors which actually characterized the animals during life.

Third.—It does not render tissues opaque. On the contrary it retains the transparency of the living parts, or may even add to it. Nerves are aften more readily traced after preservation than during life.

Fourth.—It leaves tissues as flexible as it is possible for them to be. The natural elasticity of the parts is usually perfectly retained, and brittleness never occurs.

Fifth.—It is a very convenient reagent for collectors to use. The preserving medium is a dilute solution of the commercial article in water. A collector can carry enough formaldehyde in a bottle which will slip into his coat pocket to make several gallons of the preservative. The water used in diluting it should always be that from which the collection is made, either salt or fresh, as the case may be.

Sixth.—It is a very cheap reagent. The commercial article is imported duty free by the State University of Iowa in 100-pound lots at a cost of 40 cents per pound. When made up in a 4 per cent solution the cost of a gallon is thus only 12 4-5 cents.

We might, in fact, summarize the various desirable qualities of formaldehyde as a preserving medium as being "very close to the ideal." A reagent which preserves faithfully all natural features just as they were during life. That it is infinitely

superior to alcohol is the verdict of everyone who has thoroughly tested it. It is true that it was severely criticized soon after its introduction into America, by certain workers who failed to secure *permanent* preservation with it. In all such cases of failure the solutions employed were very weak ones. A proper strength of solution is a very important detail. A solution of 4 per cent strength—that is, one containing

Commercial formaldehyde..... 4 volumes,
Water.....96 volumes,

is perfectly safe for most objects. Of course, stronger solutions are required for special cases, and slightly weaker ones for others.

Certain precautions in the use of this reagent require notice here:

First.—The gas is quite volatile, and the containing jar must be kept tightly sealed. If it be impossible to entirely prevent evaporation, changing the solution occasionally will answer perfectly well.

Second.—The solution being an aqueous one it is liable to freeze. This probably appears, at first, a very serious matter, because we are so used to alcohol as a preservative, and this does not become frozen.

Third.—The gas is irritating to the eyes, nose and throat. The effect, however, is merely temporary. Prolonged washing in water before a dissection is to be made will remove much of the reagent and reduce the annoyance to a minimum. Alcohol of 70 per cent strength appears to extract formaldehyde more rapidly than does water, but it is not always practicable to use it.

II. THE USE OF FORMALDEHYDE IN FIXING AGENTS.

In cellular biology the choice of a fixing agent means a great deal. All the conceptions which we build up about the cell appear to rest primarily upon the character of the reagent which was used in killing it. While we constantly seek to keep in our preparation the features of the living cell, how far short of the ideal we often fall every histologist knows. It is probable that certain recent investigations in cell structure will have to be gone over again because of too blind a faith in the fixing agents which were used.

Formaldehyde alone is not suitable for general cytological work. It has a tendency to produce a vacuation in protoplasm

which is very deceptive. It may, however, be combined with other reagents with superior results. When added to picric acid there is given one of the most delicate fixing agents yet imagined; one which appears to faithfully preserve every detail of structure, and which also permits of subsequent treatment in any desired way. Mixtures of formaldehyde, chromic acid, and acetic acid; or of formaldehyde, platinic chloride, and acetic acid are also very desirable. The principle involved here appears to be that formaldehyde may often be advantageously substituted for osmic acid in such mixtures on account of its superior penetration and the absence of a tendency to overfixation. In all these cases formaldehyde is to be used pure, not diluted.

III. FORMALDEHYDE IN NEUROLOGICAL WORK.

I have been impelled to make a critical examination of neurological methods in connection with a certain line of investigation in which I am engaged. Of course the technique employed in the study of any nervous system is necessarily highly specialized, but the following notes have a general application. Formaldehyde may justly claim a place in neurological methods. Its chief uses are:

First.—It is an excellent hardening agent for the brain, where anatomical methods alone are to be employed. It hardens with surprising rapidity, so that after a week or ten days a fairly large brain can be thoroughly studied.

It also preserves the form and color of the several parts. Its only undesirable effect lies in the increase in volume which is given by a solution of just moderate strength.

This tendency to swell the parts may be lessened by the use of a strong solution, one containing 10 to 20 per cent of the commercial article. It has also been recommended by various workers that a mixture of formaldehyde and alcohol be used, the tendency of the latter to shrink tissues, offsetting the swelling action of the former. Messrs. Parker and Floyd believe that they have struck the proper balance in the following mixture:

95 per cent alcohol	6 volumes,
2 per cent formaldehyde	4 volumes,

in which a barely perceptible increase in the size of the brain occurs. I believe that it is well to double the strength of the formaldehyde in this mixture, and I am accustomed to do so in my own work.

Second.—Formaldehyde has an application in those methods used for tracing the course of medullated nerve fibers. All such methods, whether the original Weigert or some modification of it, are usually long and tedious, the time required frequently being some months. This length of time is often a very serious objection. Formaldehyde can be introduced in these methods for the purpose of rapidly giving firmness to the nervous tissue, and then subsequent steps may follow in quick succession. In this way the time may be reduced to ten days for the whole process.

Third.—In the study of nerve cells formaldehyde may now claim a place in the beautiful impregnation method of Golgi. The application is made in Golgi's "rapid" method, and consists in the substitution of pure formaldehyde for the 1 per cent osmic acid of the hardening mixture. The advantages resulting from this substitution may be an increased clearness of the subsequent silver impregnation, or in the slightly wider latitude of time during which hardening may occur. The physiological condition of the nervous tissue appears to be a very important factor in all Golgi work; and perhaps formaldehyde is less sensitive to these differences than osmic acid. However that may be, osmic acid in this method cannot be dispensed with. Workers should use both hardening mixtures side by side. The results attained by one will supplement those of the other in a most valuable way, thus virtually doubling the efficiency of the study as a whole.

THE NERVE CELLS OF THE SHARK'S BRAIN.*

BY GILBERT L. HOUSER.

The sharks are of the greatest interest to the morphologist on account of the many ancestral characters of their organization. The researches of recent years indicate that they represent quite well the primitive stem of the jaw-bearing vertebrates. With this fact in mind, the importance of the study of the shark's brain is at once apparent. For obvious

* The following brief notes are to be considered as in the nature of a mere preliminary communication on this subject.