THE USE OF FORMALIN IN NEUROLOGY.

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Formalin (HCHO) is the forty per-cent. solution of formic aldehyde gas in water. The aldehyde is variously known as formaldehyde, formol and formalose, and has, of late, come into such a prominent degree of usefulness, that it might seem desirable to offer a brief, though inadequate survey of some of the uses to which it has already been put, after a year's experimentation upon neurologic material.

Formalin is prepared by subjecting methyl alcohol to oxidation.

Methyl Alcohol	+	Oxygen	=	Formaldehyde	+	Water
CH_3OH	+	O		HCHO	+	H_2O.

Further oxidation will produce formic acid.

The method of its preparation in either large or small quantities has been given by Stebbins (28). Its production in large quantities is based upon the German patent No. 55,176, issued to Auguste Trillat, Dec. 17, 1890.

It is miscible with water and alcohol in all proportions. It is kept in darkened bottles, as the light may cause decomposition or at least a separation of paraform, this may also sometimes be seen as a white substance around the stoppers of bottles; exposed to a low temperature it is said, however, that this separation has no influence on the action of formalin. Paraform does not seem to appear so readily in weak as in the strong solutions.

With various tissues, it is likely that different percentages will be useful; the percentage suitable for one form of animal may be inadequate for the proper preservation of another. Its utility as

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a preservative for laboratory specimens has been pretty well tested and found favorable. It has a neutral or slightly acid reaction and an odor resembling that of Witch Hazel; but if used in strong solutions the gas becomes very irritable to the conjunctiva and to the mucosa of the respiratory passages. Even when used in solutions diluted to two per cent.* some discomfort is caused, unless the specimens be first rinsed or soaked for a short time in water.

When the dilute solutions are used for hardening they should be occasionally renewed and kept tightly covered to prevent deterioration.

Formalin also has the advantage over alcohol of not being inflammable and of not shrinking the tissue to the same degree, nor does it destroy the natural color of the specimen so quickly; but on the other hand it is not so suitable for museum preparations which may be exposed to cold temperatures, as the amount of water present would invite freezing and consequent destruction of the jar and perhaps of the specimen.

On account of its penetrating action, large as well as small organs or specimens may be hardened in it. Its cheapness is another element in its favor; even at the rate of two dollars per pound, at which it retails in this country, it is as cheap in dilute solutions, as alcohol free of tax. In Germany it sells for four or five marks ($1.00 to $1.25) per kilo (2.2 lbs.). It furthermore possesses the advantage of dissolving certain salts more readily than alcohol, and it may therefore have a wider range of application as an adjunct in preservative methods.

A limited experience with the preparation introduced under the name of formalose indicates that it has about the same percentage of formic aldehyde as formalin and may be used in the same way.

In August, 1893, F. Blum (4) called attention to the use of formaldehyde as an antiseptic in dilute solutions. In September of the same year, the same writer (5) speaks of its action as

*When the percentage is spoken of, it refers to the proportion of *commercial formalin* present and not formic aldehyde.

a hardening medium. His attention was called to this feature from the fact that the epidermis of his fingers became hardened after working for a time with formaldehyde.

On immersing a field mouse and certain organs in a four per cent. solution, he became convinced that they were hardened as well and more quickly than when alcohol was used.

Hermann (16) finds no especial advantage nor disadvantage in the use of formalin over other fixing agents; indeed he believes that for section methods the after-treatment with alcohol is somewhat deleterious to the tissue.

F. Blum (6) discusses Hermann's paper. J. Blum (7) used formol in two per cent. solutions upon some fishes and a lizard and found them to harden in a very short time and to preserve their form and color unchanged; the latter condition being due to the fact that the mucin of the mucus-secreting animals remains transparent in formalin.

Alleger (1) states that attention was first called to the germicidal action of formic aldehyde in 1886, by Low. Gelatin is made insoluble by the formalin and this is found to be of great advantage in bacteriology and histology; in the latter it is useful as a fixative in holding the sections to the slide, by adding a few drops of the formalin for each gram of a one-half to one per cent. gelatin solution. A gentle heat is applied to the slide until the paraffin is softened and the superfluous gelatin allowed to drain from the edge of the slide. Another interesting recommendation of Dr. Alleger's is that fresh tissues may be placed directly in certain staining reagents to which have been added five per cent. of formalin, and thus hardened and stained in bulk at the same time.

Hoyer (18) has taken parts of the nervous system from corpses, hardened them in formalin and then submitted them to the Golgi method with good results.

Marcus (24) recommends hardening the spinal cord for two or four weeks in a one-half per cent. solution of formalin, then small pieces one-half centimeter thick are cut out and placed in Müller's fluid for a week in an oven at 37° C. The pieces are then dehy-

drated and imbedded in collodion, after the sections are cut they are again placed in Müller's fluid and put back in the oven from a day to a week. The sections are then quickly washed in alcohol and put in the Weigert-Pal-hematoxylin solution for at least two days.

No mention is made of the copper acetate bath, and the resulting stain is apparently due to the formation of a chromium lake more or less modified by the use of the formalin.

Strong (30) advocates the following formula for the Golgi method:

Potassium bichromate (3½%–5%), 100 Vols.
Formalin, 2½–5 Vols.

After hardening several days the tissue is transferred to the silver-nitrate solution (one per cent.). Or the tissue after one or two days may be transferred from the above bichromate-formalin mixture to the following:

Potassium bichromate (5%), 2 Vols.
Formalin, 1 Vol.

After twelve or twenty-four hours the tissue is put into the silver solution. The advantages of this method are that it avoids the use of osmic acid, and that the stage of hardening favorable for impregnation lasts longer than when the osmium-bichromate mixture is used. In other words the formalin-bichromate does not over-harden. In this respect it is superior to the lithium-bichromate method of the same author. For embryonic tissue he finds the osmium-bichromate preferable.

Van Gieson (31) has used formalin in four per cent., six per cent. and ten per cent. solutions for ordinary histologic methods, followed by ninety-five per cent. alcohol and collodion imbedding. Weigert's hematoxylin method can be applied to such sections and gives very good results for the plexus of fine fibers in the cortical and spinal gray matter. The myelin of the fine fibers is well preserved and gives the characteristic blue-black reaction with the Weigert hematoxylin stain, as in chrome-hardened preparatious. The background of the gray matter is especially clear and the fibers

sharply delineated. The formalin-hardened sections should be soaked in the neutral copper-acetate solution diluted one-half with water for two hours, then thoroughly washed in water and immersed in the Weigert lithium-carbonate-hæmatoxylin solution from four to twelve hours. Weigert's borax-prussiate-of-potassium solution is useful for differentiation.

In the absence of any chrome salts in the above method, the stain is the result of the formation of a copper lake. The method has been confirmed in our own laboratory and the results were all that could be desired.

Dr. Van Gieson also finds that formalin-hardened sections are useful in Rehm's modification of Nissl's method, but that the minute structure of the nucleus and cytoplasm is not quite so sharply outlined as with fixation in absolute alcohol. The duration of the hardening period in formalin exerts an important and varying influence upon the tissues. Further investigations upon this matter are promised.

René Marie (25) uses a one per cent. solution of formalin and allows it to harden the tissue for four or five days. The usual staining methods are employed.

Lachi (21) finds that formalin in weak (one to two per cent. solution) or in stronger solution (ten to fifteen per cent.) exerts an injurious influence upon connective tissue by dissolving the fundamental substance, especially in the elastic fibers and mucosa. If used for such tissue some corrective should be employed.

He finds it of signal service in the nervous system, either in the central or peripheral, or in the embryo or adult. Nerves kept for a few days in from two to five per cent. solutions can be treated with silver nitrate, and show the characteristic cross of Ranvier. Pieces from the central nervous system treated from five to nine days in a mixture of equal parts of twenty per cent. formalin and six per cent. potassium bichromate, gave the black reaction with silver nitrate equally as well as the osmium bichromate mixture with the advantage that the blackness of the tissue was not so great as when osmic acid is used. Favorable results were obtained from the myel and embryonic brain of cows, and

with the cerebrum, cerebellum and myel of the human adult.

The mixture indicated above is also recommended for the Weigert method. Paraffin is not advocated for an imbedding medium on account of friability.

Kenyon (20) records a free translation of J. Blum's article in the Bericht, über d. Senckenbergische naturf. Gesell. in Frankf., a. M., 1894, and adds valuable observations of his own. Blum states that formaldehyde was discovered in 1863 by A. W. Hoffman while passing wood spirit (methyl alcohol) and air over a red-hot platinum spiral.

The vapor carried into water to the point of saturation gives a forty per cent. solution of formaldehyde. This Blum calls formal because it was known under that name when it was first used in aqueous solution for disinfecting, hardening and preserving.

A reference is made to Born : " Demonstrationen einer Anzahl in Formaldehyde (Formol) gehärteter menschlisher Gehirne. Mediz. Sektion der schlesisch. Gesell. f. vaterl. Kultur, 1894," stating that pieces or even the entire brain hardens quickly, and the white and gray matter are sharply differentiated.

Blum also performed interesting experiments in preserving hens' eggs, certain invertebrates, vertebrates, fruits and plants.

Kenyon experimented upon a variety of forms and found a four per cent. solution of formic aldehyde (ten per cent. of the commercial formalin) best adapted to Salamanders.

Different percentages were tried; the lowest being one-fourth per cent. and the highest twenty per cent., some of the dilute solutions were found useful for some of the invertebrates. The fact noted by F. Blum was verified, namely : that the vessels containing blood lost color when hardened in formalin, but this reappeared when treated by alcohol. This was explained by the coagulation of the fibrin by the alcohol giving it a yellow color and making it opaque and by bringing the corpuscles again to view. In conclusion, Mr. Kenyon believes that a solution stronger than two per cent. of the formalin is necessary to prevent the swelling and decolorization of specimens, and that from four to eight per cent. will give the best results.

To counteract the swelling caused by the weak solutions of formalin, alcohol was added. For histologic purposes a mixture of alcohol and formalin was found to act better than either one used alone.

Durig (11) also employs formalin as a substitute for osmic acid in the silver-impregnation method. His plan is to harden a piece one-half centimeter square for three days in four to six per cent. of formalin and three per cent. Potassium bichromate, then dry off on filter paper and immerse in a three fourths per cent. silver solution. After two days return to the first mixture—and lastly, immerse it in silver containing a trace of formic acid.

Stebbins (28) gives a good description of the chemistry and preparation of formalin. This agent has recently been introduced into photography for hardening gelatine films, and found to be of great service. Formalin is a powerful reducing agent. In aqueous solution it reduces ammoniacal nitrate of silver to metallic silver, forming a mirror on the sides of the vessel containing the solution. It unites with bisulphate of soda or potassium, to form a crystalline addition product. This reaction may advantageously be used for separating formaldehyde, as well as homologous aldehydes from mixtures of other bodies.

The combination of formalin with other hardening reagents apparently has not, as yet, received much attention ; its use in this connection will undoubtedly be of great value in macroscopic as well as microscopic methods.

Experiments with formalin show that good results may be obtained with nervous tissue when the following mixture is employed :

Water, . . .	2000 cc.
Formalin, . . .	50 cc.
Sodium chloride, .	100 grams.
Zinc chloride, . .	15 grams.

The specific gravity should be about 1.05. In practice the brain is left in this mixture for a week or ten days (a longer stay is not detrimental) and when practicable the cavities and blood vessels are injected with the same mixture in order to insure a

more uniform hardening. The specimen may then be transferred to 2½ per cent. formalin (water, 2000 cc., formalin, 50 cc.) and may remain in this solution indefinitely if the jar be kept tightly covered; or if it is to become a museum specimen it may, after a week in the second solution, be removed to fifty per cent., seventy per cent. and 90-95 per cent. alcohol for final storage.

An objection to the use of formalin solutions for museum purposes would be the large proportion of water present, which would freeze at low temperatures and cause injury to the specimen or jar containing it. Since formalin is readily miscible with alcohol, as well as water, enough of the former might be added to prevent the freezing, say equal parts of ninety-five per cent. alcohol and two and a half per cent. formalin; the exact proportions have not as yet been determined.

After an immersion of two weeks in the formalin solutions, a human brain lost only 6.8 per cent. of its weight; but after an immersion in fifty per cent. alcohol for eight days and an immersion for an equal length of time in seventy per cent. alcohol, a total of sixteen days, it was found to have lost twenty-two per cent. of its first weight. A monkey brain after an immersion of eight days in the formalin mixture lost 5.4 per cent. of its weight; continued immersion in the same fluid for eighteen days longer caused a loss of less than two per cent. A fox brain was immersed in a similar mixture for five days and lost 6.5 per cent. of its weight; it was left in the same mixture eighteen days longer and lost 2.3 per cent. more of its weight. The brains were firm and in excellent condition for dissection.

The second, or two and one-half per cent. formalin-solution redissolves any of the sodium chloride that may remain in the brain, which is an advantage if the specimen is to be treated with alcohol, as the latter does not dissolve the salt. The brain should not be put from the formalin solution immediately into the strong alcohol as the tissue will shrink very materially.

Material treated in the way above described has yielded most satisfactory results histologically. Portions of the central nervous system of an adult, after treatment with the above brain

mixture, have been later treated with other fixing reagents : *e. g.* corrosive sublimate, picro-aceto sublimate and a chrom-acetic-acid mixture with most excellent results. Equal parts of a two and-one-half per cent. solution of formalin and the picro-aceto sublimate proved very satisfactory. The same proportion of formalin with the chrom-acetic mixture worked very well ; but the combined mixture turned green after a short exposure to the light.

On November 3, 1894, in working on the myel of a young kitten, I substituted formalin for the osmic acid in the Golgi-Cajal method, using the following formula :

Potassium bichromate, 3%, 4 Vols.
Formalin, 2%, 1 Vol.

The specimen was left in the mixture for nine days, then imbedded and cut ; the results were very promising. In later experiments I added the strong formalin directly to the bichromate solution.

Formalin, 2 cc.
Potassium bichromate 3%, 100 cc.

The specimens remained in the mixture for three days and an equal length of time in the three-quarter per cent. silver solution.

The impregnated cells with their processes were particularly distinct, standing out like black diagrams on a clear back ground. After a time, although the cells and processes remained distinct, the action of the light caused the sections to lose their light color and turn yellowish brown, resembling very much the sections prepared by the osmium-bichromate method.

The following mixture was also tried with even greater success than the preceding :

Müllers fluid, 100 cc.
Formalin 10%, 2 cc.
Osmic acid 1%, 2 cc.

The formalin-bichromate mixture was not tried upon embryonic tissue, nor the brains of low or generalised forms.

Care must be taken to keep the formalin bichromate mixture in the dark to prevent its deterioration. Exposure to the light

causes the solution to assume a dark muddy color, the result of some chemical change. It is advisable, therefore, to mix the two solutions as they are needed and keep the mixture concealed.

Gage (15) has tested formalin as a dissociating agent. He recommends the following formula :

Normal salt solution, 1000 cc.
Formalin, 40%, 2 cc.

His results were highly satisfactory. The solution acts quickly and yet retards deterioration for some time.

After three hours the ciliated cells from the trachea of a kitten were easily separated upon a slide and almost as good preparations were obtainable after ten days. The endymal cells bordering the encephalic cavities were found to be very susceptible to the mixture, also " some of the cerebral cortex from various regions was tested, and it was found comparatively easy to obtain excellent preparations in which many of the multipolar nerve cells were wholly isolated."

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