

FREEBORN (G.C.)

A RÉSUMÉ OF
THE USES OF FORMALIN.

BY

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A *RÉSUMÉ* OF THE USES OF FORMALIN.*

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THIS reagent is also known in commerce under the names of formol and formalose. It is a forty-per-cent. solution of the gaseous body, formic aldehyde (HCOH), in water. It is prepared by oxidizing methylic alcohol and bringing the resulting gas into solution in water. It is non-inflammable. It mixes in all proportions with alcohol and water. Its power of penetration is good. Its keeping properties are good. A series of experiments were instituted to determine this point, with the following results: A forty-per-cent. solution was kept in open and closed vessels, daily tests being made. The results of these experiments showed that the solutions did not decompose. There was a loss of 1.6 per cent. of formalin and an increase of 0.1 per cent. of formic acid. Polymerization took place with the formation of a butterlike mass containing sixty per cent. of formic aldehyde; this dried up into a hard mass which contained eighty-five per cent. of formic alde-

* Read before the New York Pathological Society, March 23, 1896.

hyde. Fish and others advise that it be kept in darkened bottles, as the light may decompose it. In an experience of two years I have not noted any appreciable change in the solutions.

Attention was first called to the antiseptic properties of formalin by F. Blum, in 1893. In 1894 Pottevin found that when formalin was added to cultures of bacteria, their growth was arrested. Cohn also found that solutions and the vapor of formalin killed bacteria both in the vegetative and in the spore stage, but that it had but little action on molds unless used in strong solutions. This want of action on molds has also been noted by many other observers.

Miquel, in experimenting with gaseous formic aldehyde, found that it acted as a disinfectant for small and loose objects confined in small spaces, but was not reliable for disinfecting large rooms.

Cambier and Brochet also experimented with the gaseous form, with results similar to those of Miquel. Their laboratory experiments were satisfactory, but their attempt to disinfect a large room did not give perfect results. They, however, demonstrated the fact that layers of dust a centimetre thick were rendered sterile. They also devised a portable apparatus for producing the gas.

Alleger made quite an extensive series of experiments in order to determine the germicidal action of formalin on bacteria. He made use of cultures of the bacillus of diphtheria in Petri dishes. The surfaces of these dishes were sprayed with solutions of formalin varying in strength from 1 to 10,000 to 1 to 100. He found that a solution of 1 to 2,000 prevented the growth of the bacillus but not that of molds. Another series of experiments were made with stick cultures in test tubes. Five drops of solutions of formalin, varying in strength from 1 to 20,000 to 1 to 100, were placed on the surface of the culture media in each

tube. At the end of forty-eight hours none of the tubes which had been treated with a 1-to-100 or stronger solution showed any growth. A third series of experiments were made with smear cultures, which were allowed to grow for from twenty-four to forty-eight hours and then they were treated with the above mentioned solutions of formalin for a few minutes. Cultures were then made from these, with the result that no growth took place from those treated with the stronger solutions.

Formalin has also been used in surgery, obstetrics, and gynæcology as an antiseptic, but has had to be abandoned on account of its irritating properties.

As a preserving agent formalin was first used by the botanists. Cohn experimented with it extensively, and found that the green and red colors of plants were not extracted. At the end of five months his specimens still retained their natural colors and were not shriveled. The botanists Sadebeck and Holfert recommend it highly.

It was first introduced into the zoological technique by F. Blum, who obtained excellent results with it as a preservative agent, and it has now come into general use.

The excellent results obtained by the botanists and zoologists with formalin as a preservative soon resulted in its introduction into the anatomical and histological technique, and at the present time it is quite generally used.

As a preservative agent for gross specimens, it is used in the strength of two per cent.,* though weaker solu-

* Bolles Lee (*Anat. Anz.*, xi, 1895, p. 253) calls attention to what he considers an inaccurate use of the terms formol, formalin, and formaldehyde; also to the manner of stating the percentages used. He maintains that the proper way of stating the strength of the solutions is to say "formol or formalin diluted with so many volumes of water."

Parker and Floyd (*Anat. Anz.*, xi, 1896, p. 567) reply to the criticism made by Bolles Lee in the above-cited article. They contend

tions, from three quarters to one per cent., have been used. These weaker solutions are objectionable on account of the likelihood of the growth of molds, and because they cause more or less swelling of the tissues. As the result of the experience of numerous observers, it appears that five-per-cent. solutions give better results.

The quantity of the solution should be large—a hundred times the volume of the specimen—and the fluid should be renewed at the end of twenty-four hours. In some cases it is well to renew the fluid a second or even a third time.

Formalin used in this manner preserves the natural form, the transparency, and, to a certain extent, the natural color of the specimens. In some specimens the blood-color appears to be bleached out, but if the preparation is placed in strong alcohol this is nearly if not entirely restored.

For preserving the blood-color of specimens, Johres makes use of the following procedure and fluid :

| | |
|-------------------------|----------|
| Sodium chloride..... | 1 part; |
| Magnesium sulphate..... | 2 parts; |
| Sodium sulphate..... | 2 “ |
| Water..... | 100 “ |

To this mixture are added from five to ten parts of a forty-per-cent. solution of formalin. After the specimen has become sufficiently hardened, pour off the formalin solution, wash the specimen in ninety-five-per-cent. alcohol, then keep it in ninety-five-per-cent. alcohol until the blood-

“that for the sake of consistency the same method of expression ought to be used for alcohol—*i. e.*, ninety-five volumes of alcohol and five volumes of water. These expressions seem to us unnecessarily cumbersome, and as they are in no way more precise or less ambiguous to one familiar with the meaning of per cent. than the expressions we used, we prefer them.”

color becomes restored, and finally preserve it in a mixture of equal parts of glycerin and water.

Fish makes objection to the use of formalin as a permanent preservative on account of the large amount of water present, which might cause freezing, and advises the addition of an equal volume of alcohol. Hodenpyl,* in using formalin for making sections on the freezing microtome (see below), found that the least trace of formalin left in the specimen prevents its freezing. It would therefore seem that Fish's objection is not valid.

Koehler and Lumière found that if from fifty to a hundred and fifty cubic centimetres of a solution of one volume of formalin diluted with four volumes of water were injected into the gastro-intestinal canal of small animals by the mouth and anus, also into the carotid artery, and the animal was kept hung up in the air, in a dry place, for some weeks, it was perfectly preserved without distortion. They performed an autopsy on an animal—a guinea pig—treated in this manner, four months after, and found the tissues and organs perfectly preserved. Dr. Henry Power † has treated the bodies of children in a manner similar to this with good results.

Professor George S. Huntington informs me that he has used formalin for the preservation of organs. He injects a solution of from two to twenty-five per cent. into the blood-vessels, and the result is a perfect preservation of the form and color of the organ. He has found that it is of no use for preserving dissecting material.

For the preservation of brains formalin has given excellent results. The fresh brain is placed in a ten-per-cent. solution, and at the end of ten days it will have sufficiently hardened to permit of the making of thick sections for demonstration of the gross anatomy, the distinction be-

* Personal communication.

† *Ibid.*

tween the white and gray matter being more sharply defined than when alcohol is used.

Parker and Floyd confirm the observations of Lanzilotti-Buonsanti, Hoyer, Hoffer, and others, in regard to the swelling of the brain when formalin alone is used. In a sheep's brain they found this swelling to be forty per cent. of its original volume. In order to correct this defect they experimented with various reagents in combination with formalin. They finally found that a mixture of six volumes of ninety-five per-cent. alcohol and four volumes of a two-per-cent. solution of formalin gave nearly perfect results. Sheep's brains hardened in this mixture retained their original color and form, and were very little increased in volume. "A brain that before treatment (June 20th) measured one hundred and one cubic centimetres, when finally prepared (July 15th) measured one hundred and three cubic centimetres."

Fish states that an excellent hardening of the brain may be obtained with the following mixture:

| | |
|----------------------|--------------|
| Water | 2,000 c. c.; |
| Formalin | 50 " |
| Sodium chloride..... | 100 grms.; |
| Zinc chloride..... | 15 " |

The specific gravity should be about 1.05. The brain is left in this mixture for a week or ten days. The blood-vessels and cavities should be injected with the fluid if possible. After the end of the ten days the brain is transferred to formalin, 50 cubic centimetres, and water, 2,000 cubic centimetres, where it may be kept indefinitely; or, after being a week in this fluid, it may be first transferred to fifty-per-cent., then to ninety-per-cent., and finally to ninety-five-per-cent. alcohol. He has also treated portions of the adult central nervous system by this method, and

afterward with mercuric chloride, picro-aceto-sublimate, and chromacetic-acid mixtures, with good results.

For hardening eyes Leber used formalin mixed with water in the proportion of one to ten. The natural color and transparency of the organ were retained. The cornea and lens became but slightly cloudy. In his opinion, the fine structure was as well preserved as with Muller's fluid. If the eyes were placed in alcohol the cornea and lens became opaque. I have employed formalin in a five-per-cent. solution for this purpose with the same results.

As a hardening agent for microscopic work, formalin has been used very extensively, the strength of the solutions employed varying from one per cent. to the full strength—forty per cent. As the results of many observations, it may now be said, with possibly one or two exceptions, that formalin alone is an unfit reagent for hardening tissues for microscopic work. It was condemned by Hermann in 1893; Lachi states that it has an injurious effect on connective tissues, smooth and striated muscle, and embryos. Many other observers condemn its use without being so specific as Lachi.

The exceptions, where it gives satisfactory results, are mucous membranes and the central nervous system. I have used it in five-per-cent. solution for hardening cystic adenoma of the ovary, with good results; also for the mucous membrane of the uterus.

Lachi, who has condemned its use for all other tissues, speaks well of its action on the central nervous system.

Van Gieson has employed it in four-, six-, and ten-per-cent. solutions for hardening the central nervous system. The ganglion and nerve fibres were well fixed. Sections stain well with Weigert's hæmatoxylin method. He has also used it for hardening the central nervous system for

after-staining with Rehm's modification of Nissl's method. The results were good, but not quite so sharp as with alcoholic hardening.

The best results for microscopic work are obtained when formalin is combined with other fixing reagents. When it is used in combination with the chrome salts more rapid penetration is obtained, whereby the time required for hardening is shortened. I have used a solution of formalin in Müller's fluid made as follows :

| | |
|--------------------------------------|-------------|
| Potassium dichromate..... | 2 grammes ; |
| Sodium sulphate..... | 2.5 “ |
| Two-per-cent. solution of formalin.. | 100 c. c. |

With this fluid I have obtained excellent preservation of the ovary, the uterus, etc. At the end of forty-eight hours the specimen is cut into slices an eighth of an inch thick ; these are washed in water for two hours ; they are then placed in alcohol for twelve hours, and then carried through the usual processes of imbedding in celloidin. Specimens hardened in this manner show no shrinkage, and the tissue elements are well preserved.

Landowsky recommends the following fixing fluids for mitotic figures in cells :

| | | |
|-------------------------------------|-----|---------|
| 1. Water..... | 20 | c. c. ; |
| Alcohol (ninety-five per cent.).... | 10 | “ |
| Formalin..... | 3 | “ |
| Hydric acetate..... | 0.5 | “ |
| 2. Water..... | 30 | c. c. ; |
| Alcohol (ninety-five per cent.).... | 15 | “ |
| Formalin..... | 5 | “ |
| Hydric acetate..... | 1 | “ |

Probably the most successful use of formalin in histological technique is its substitution for osmic acid in the osmium-dichromate fluid used in Golgi's silver method for the central nervous system.

This substitution was probably first made by Dr. O. S. Strong, though it has been recommended by Lachi and others. Strong employs the following mixture:

| | |
|---|----------------|
| Potassium dichromate (3.5- to five-per-cent. solution)..... | 100 vols.; |
| Formalin..... | 2.5 to 5 vols. |

After the specimen has been in the solution for several days it is transferred to a one-per-cent. silver-nitrate solution; or at the end of two days it is transferred from the formalin-dichromate mixture to the following:

| | |
|---|----------|
| Potassium dichromate (five-per-cent. sol.)... | 2 vols.; |
| Formalin..... | 1 vol. |

After remaining in this fluid for from twelve to twenty-four hours it is placed in the silver solution. The advantages of this method are, that the stage of hardening is prolonged, the stage favorable to impregnation lasts longer, and the results are more certain. For embryonic tissue he does not consider it as good as the osmic-dichromate mixture.

Fish has used the above-described method, but thinks he has obtained better results with the following:

| | |
|---------------------------------|------------|
| Müller's fluid..... | 100 c. c.; |
| Formalin (ten per cent.)..... | 2 " |
| Osmic acid (one per cent.)..... | 2 " |

Strong has also used formalin as an injection medium for hardening brains *in situ*. He uses formalin diluted with an equal volume of water. This he injects into the cerebral vessels until it runs out of the cut jugulars. After a few minutes he makes a second injection, then a third, and even a fourth, at intervals of fifteen minutes. The brain is then removed from the cavity of the skull. The swelling which usually occurs when formalin is used does not take place. Sections from brains hardened in this manner may be stained by either the Weigert or the Golgi method.

When the Golgi method of staining only is to be used an equal volume of a ten-per-cent. solution of potassium dichromate is added to the formalin in place of the water.

Dr. T. S. Cullen has devised two methods for using formalin in connection with frozen sections. They are as follows:

METHOD I.

1. Keep sections made with the freezing microtome in a five-per-cent. aqueous solution of formalin for three to five minutes.

2. Keep them in fifty-per-cent. alcohol for one minute.

3. Keep them in absolute alcohol for one minute.

4. Wash them in water.

5. Stain them in hæmatoxylin for two minutes.

6. Decolorize them in acid alcohol (1.5 per cent. HCl).

7. Wash them in water.

8. Stain them with eosin for twenty seconds.

9. Place them in ninety-five-per-cent. alcohol.

10. Pass them through absolute alcohol, clear them in creosote or oil of cloves, and mount them in Canada balsam.

The blood being lost in the frozen sections, the defect was overcome by fixing the tissue in formalin, and then making frozen sections as in

METHOD II.

1. A piece of tissue $1 \times 2 \times 5$ centimetres is kept in a twenty-per-cent. aqueous solution of formalin for two hours.

2. Frozen sections are made.

3. Keep them in fifty-per-cent. alcohol for three minutes.

4. Keep them in absolute alcohol one minute.

5. Wash them in water and stain them in hæmatoxylin for two minutes.
6. Decolorize them in acid alcohol (1·5 per cent. HCl).
7. Wash them in water.
8. Stain them in eosin for twenty seconds.
9. Place them in ninety-five per-cent. alcohol.
10. Pass them through absolute alcohol, clear them in creosote or oil of cloves, and mount them in Canada balsam.

Method I is used for diagnosticating bits from tumors, and it is possible to make a report in fifteen minutes. Method II is used mostly for the examination of uterine curettings. The author's practice is to have bottles containing a ten-per-cent. solution of formalin in the operating room. The curettings are immediately placed in one of these, and by the time they reach the pathologist they are hard enough to make frozen sections of.

Bender has also used formalin for making frozen sections, not for preliminary hardening, as in Cullen's method, but for completing the hardening of specimens that have already been in alcohol. He places pieces of tissues, two millimetres thick, that have been in alcohol, in a one-per-cent. solution of formalin, and keeps them there until the alcohol is completely removed. This requires from half an hour to an hour. He then washes them well in water and makes frozen sections. The tissue, he states, is rendered soap-like in consistence by the action of the formalin.

Ohlmacher states that formalin, when used in from two- to four per-cent. solutions, acts as a powerful mordant for aniline dyes. Cover-glass preparations are treated for one minute with the solution, washed well in water, and then stained in the cold. Or it may be used instead of aniline oil or carbolic acid as a menstruum for dissolving the dyes. One gramme of fuchsine or other aniline dye is dissolved in ten cubic centimetres of alcohol, and this is added to one

hundred cubic centimetres of a four-per-cent. solution of formalin. Formalin methylene blue, made by dissolving one gramme of methylene blue in one hundred cubic centimetres of a four-per-cent. solution of formalin, makes an effective stain. A saturated solution of safranin in a four-per-cent. solution of formalin gives a beautiful double stain when used after the formalin methylene blue. Nuclei stain blue, plasma stains reddish.

S. H. Gage has used the following solution as a dissociating agent with good results :

| | |
|---------------------------------|---------------|
| Normal salt solution..... | 1,000 c. c. ; |
| Formalin (forty per cent.)..... | 2 " |

Formalin has been used by Hauser for preserving plate and tube cultures of bacteria. His method is as follows: Plate cultures, in Peri's dishes, have a piece of filter paper placed under the cover, which has been moistened with ten to fifteen drops of formalin. The plates are then placed in a closed vessel in the bottom of which is laid paper or cotton saturated with formalin. After twenty-four hours the cultures are fixed. Test-tube cultures are closed with a plug of cotton that has been wet with formalin and then placed in a closed chamber as above. After twenty-four hours they are removed and sealed with sealing wax, when a permanent preparation is obtained. Colonies from plate cultures may be permanently mounted by the following procedure: The selected colony is cut out of the plate and placed on a slide and covered, and then a little of the melted medium is run under the cover. The slide is then exposed to the action of the vapor of formalin for twelve hours. Formalin renders ordinary culture media, gelatin, and that fluidified by bacteria non-liquefiable by heat. The above-mentioned method of preserving bacteria has been employed successfully by Alleger, Cheesman, and many others. I am informed by Dr. Cheesman that cultures treated in

this manner by him a year ago are still well preserved, but some of the chromogenic forms have lost their color to some extent.

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