

*Prof. G. Gosson. with the Author's  
Compliments*

Reprinted from "NATURAL SCIENCE," Vol. VII., No. 46, Dec., 1895.

*151494*

# The Use of Formalin as a Preservative Medium for Marine Animals.

BY

JAMES HORNELL.







## The Use of Formalin as a Preservative Medium for Marine Animals.

EARLY in the present year, my attention was drawn to the use of Formalin as a preservative fluid, by a short account of some experiments carried on by Professor Paulino de Oliveira<sup>1</sup> at the University of Coimbra. Since then, I have conducted in the laboratory of the Jersey Biological Station a series of very extensive tests, having the action of the fluid in question under daily observation. As the original investigation was conducted by Professor de Oliveira on rather restricted lines, and further, as my results differ considerably from his, it may be useful to workers at other stations and elsewhere if I bring together the chief of my methods and state the principal results obtained.

As to the nature of the fluid, it is sufficient to mention that formalin is the name given to an aqueous solution containing 40 per cent. of formic aldehyde. Its chief commercial use at present is as an antiseptic, and from its great bactericidal powers it takes high rank as such.

As a fluid designed to preserve marine organisms permanently, formalin is best used in an aqueous solution of strength ranging from two to eight per cent., and while a two per cent. solution will in many cases give satisfactory results, in practice I find it advantageous, in order to avoid any risk, to employ no solution of less strength than three per cent. Where the animal is bulky, or contains much water, I prefer to use a five per cent. solution. Hence in the following notes, unless otherwise stated, the strength employed must be understood to be that last named. It is to be noted that these percentages are calculated by considering the commercial fluid, formalin, as being of full strength (100 per cent.), although really it is but a 40 per cent. solution of the active principle.

For convenience in referring to these methods, I will treat of the various phyla and classes separately—I will also premise that the use of formalin does *not* do away with the employment of the ordinary fixing agents if the object is wanted for histological purposes. For dissection and museum purposes, there is, however, no need of other fixation than the action exercised by the formalin itself.

<sup>1</sup> *Annaes de Sciencias Naturaes*, vol. 2, no. ii., p. 69. (Porto; 1895.) (See also *NATURAL SCIENCE*, vol. vi., p. 229, April; 1895.)

PROTOZOA.—Perfect preservation, with cilia distinctly shown, is attained in a six per cent. solution. For microscopical fluid-mounts of these organisms, carbolised water and allied fluids may be advantageously discarded for this new medium, and by the use of aqueous staining fluids containing a percentage of formalin, good differentiation can be obtained; weak bismarck brown is one of the best stains to employ.

PORIFERA.—For histological details, formalin preparations are inferior to such as have been thoroughly well fixed and passed carefully into spirit, but the former are infinitely superior to such spirit specimens as have not had the *utmost* care bestowed upon them. The same observation applies fairly universally to other groups.

For ordinary dissection, formalin preparations are equal to the most carefully prepared spirit ones; while for museum preparations, those in formalin are greatly superior, showing absolutely no contraction, and in those where delicate filmy tissue is present the lovely transparency of life is retained almost unimpaired.

HYDROIDEA.—For microscopical manipulation, spirit is superior, as, when staining is employed after subsequent grading into alcohol, there is found to be a lack of differentiation not present in specimens prepared in the ordinary way. As a killing agent, I have, however, successfully employed formalin upon the Gymnoblasic Hydroids, and find that imperfect staining can be obviated by the immediate transference of the animals, after death in the formalin, to an ordinary fixative, with subsequent grading into spirit.

For dissecting and museum preparations, formalin is much more satisfactory than spirit, giving exquisite transparency, very marked in contrast with the dull opacity of spirit-preserved specimens. Specially fine are the fleshy medusæ of the *Sarsia* type, preserved by simple transference, while alive, into the fluid.

For the Calyptoblastic Hydroids, formalin has no special advantage.

ACTINOZOA.—These, as a whole, give excellent results in formalin, provided they be kept in not less than six per cent. strength. For those with very thin-walled tentacles, *e.g.*, *Tealia crassicornis*, a seven per cent. or even an eight per cent. solution is preferable; preparatory hardening in corrosive sublimate is also beneficial, but this somewhat impairs the transparency.

DISCOMEDUSÆ.—The larger medusæ have long been considered well-nigh the most difficult of organisms to preserve satisfactorily. By the employment of formalin, all difficulty disappears, as after a number of experiments this summer I discovered that simple immersion in a five per cent. solution is all that is necessary. To-day, after the lapse of six months, the specimens are as good as, and indeed considerably tougher than, the first day they were placed in the medium. They (*Aurelia* and *Rhizostoma*) are glassy transparent, with the radial canals showing conspicuously in a delicate opalescent white.



The Lucernariidæ—except for histology—should always be preserved in formalin, the results are so good. In this case, previous stupefaction is necessary.

CTENOPHORA.—*Cydidippe* and *Beroë* are not satisfactory in formalin; they seem unable to attain sufficient toughness to allow them to sustain their own weight in the fluid.

ECHINODERMATA.—Spirit and formalin are about equal in results.

ENTEROPNEUSTA.—*Balanoglossus* is worthless in formalin, as it secretes too great an amount of mucus, and spirit is necessary to coagulate this.

VERMES.—The Turbellaria can only be preserved in spirit. Nemertines and Polychætes, if previously well fixed, are very satisfactory in formalin, but the use of 50 per cent. spirit fortified with 5 per cent. formalin is even better. Such, however, of the Polychætes as secrete much mucus, e.g., *Chaetopterus*, must be preserved in the ordinary way by passing into strong spirit. *Formalin has little or no hardening action upon mucus.*

Some of the smaller Nemertines are killed very well extended by being thrown into formalin.

Rotifers preserve beautifully in formalin if previously fixed in corrosive sublimate.

POLYZOA.—For fleshy forms, such as *Alcyonidium*, formalin is most useful, obviating the dull opacity of spirit specimens.

CRUSTACEA.—For all except those with a well-marked carapace (*Leptostraca*, *Thoracostraca*, etc.), an 8 per cent. solution gives beautiful results, transparency and pliability. Where, however, a carapace is present, this frequently rises, giving an unnatural gap between the posterior margin of the carapace and the succeeding segment. For museum purposes, this is unsightly, but for dissection work there is the counterbalancing advantage of giving the preservative fluid freer admission to the organs beneath the carapace—always a difficult point in the preservation of crustacea.

MOLLUSCA.—Almost without exception formalin is vastly superior to spirit for these animals, and ordinary fixing is quite immaterial—without becoming intensely hard, as they do in spirit, an agreeable firmness, very useful in dissection, is produced. While previous anæsthetising is in no way superseded in the majority of cases, the cephalopods are simply magnificent if transferred direct to a 4 per cent. solution, and I dare prophesy that when once these formalin preparations are seen by our museum authorities there will be a general turning out of the old spirit specimens.

TUNICATA.—Very good and natural results are here obtained if the animals be previously thoroughly stupefied. A weak solution of formalin must be avoided with the delicate species, say anything less than a 6 per cent. strength. *Botryllus* in spirit is a depressing object-lesson of how not to preserve naturally, and though the greater part of the colour fades in formalin, yet the way in which all distortion of the

parts through shrinkage is avoided gives a life-like appearance as satisfactory as any that can be hoped for.

FISHES.—For these a 4 per cent. solution is ample to produce the finest results.

*Amphioxus* is best killed by plunging direct into the fluid. The result is most beautiful; the natural transparency is so completely retained, that the internal organs can easily be traced, and the buccal cirri protrude gracefully as in life, and not in the state of tangled retraction seen in spirit specimens. Shrinking is quite obviated, and until one can compare a formalin prepared specimen with one fixed and preserved in spirit in the ordinary way, it is impossible to comprehend how much shrunken the latter are.

Elasmobranchs, such as *Scyllium*, are also beautifully preserved for dissection by simple immersion in a 4 per cent. solution of formalin, provided the viscera, heart, and brain be freely exposed by cutting away the abdominal wall and opening the pericardium and brain case. In two days the viscera attain the consistency of gutta-percha. Objectionable smell is quite obviated, the muscles assume a snowy whiteness, and the preparation as a whole becomes so clean and sweet that it is a real pleasure to dissect it. Anyone who has dissected an old-time *Scyllium* can understand what an improvement this means.

RECAPITULATION OF RESULTS.—For histological details, preservation by simple immersion in a strong solution of formalin gives fair results; prior fixation by one of the accepted and appropriate methods gives even better, but both are unmistakably inferior to those produced by fixing and grading into spirit in the ordinary way.

A minor and very useful employment of formalin in microscopical technique is, I find, to add 3 per cent. to aqueous staining fluids, to obviate any chance of the maceration of objects placed therein. Again it may be employed as a 3 per cent. solution, in place of pure water, in the washing out of ordinary fixatives. I have known many valuable preparations spoiled in the washing out, some by being inadvertently left in too long, and others, again, deteriorated through lack of sufficient washing, due to a fear of possible maceration. By the employment of a formalin washing solution, ample time can be allowed for getting rid of the fixative without the least fear of maceration, as, even if the preparation be left washing longer than intended, the formalin will prevent any ill effects.

For dissecting and museum specimens, simple immersion in formalin solution gives, except in the case of *Ctenophora*, *Turbellaria*, *Chaetopterus* and allied worms, *Balanoglossus*, and a few others, results not inferior to those obtained by ordinary fixation and grading into strong spirit, while in the majority of cases the results are greatly superior.

Professor de Oliveira believed that formalin would preserve natural colouring little impaired, but my prolonged experiments negative this, for while the loss of colour proceeds much more slowly



than in spirit, still it is but a question of time ere it vanishes. This extraction is most marked and rapid in the highly-coloured sponges and tunicates. Crustaceans, however, retain their colours well.

In addition to the foregoing, I have further experiments with formalin in progress, and trust to present the results at an early date.

Taking every consideration into account, formalin may be regarded, for the majority of purposes, as superior to spirit in the results obtained, and when we remember its greater cheapness—a gallon of strong solution costs on an average but 1s.—and the ease with which we can apply it, it being miscible with water, spirit, and other fluids in any proportion, we may account its introduction as one of the greatest services ever rendered to the working biologist, while to the naturalist in remote places, to whom restricted baggage is a paramount consideration, formalin in its concentrated form will prove an inestimable boon, bringing the formerly unattainable within easy reach—all to the enrichment of our great museums and the advancement of zoological knowledge.

JAMES HORNELL.









# NATURAL SCIENCE:

A Monthly Review of Scientific Progress.

The following are a few **FACTS** as to the work of  
**"NATURAL SCIENCE"** during 1895.

## NATURAL SCIENCE FOR 1895 HAS PUBLISHED CONTRIBUTIONS FROM:

ABBOTT, W. J. Lewis, F.G.S.	HERDMAN, Prof. W. A., D.Sc., F.R.S.	PELSENEER, Prof. Paul, D.Sc.
ANDREWS, F. W., M.D.	HICKSON, Prof. S. J., M.A., D.Sc., F.R.S.	PERCIVAL, Prof. J., M.A.
ANDREWS, C. W., B.A., B.Sc., F.G.S.	HOEK, Dr. P. P. C.	POCOCK, R. I.
BARBER, C. A.	HORNELL, James.	POPE, W. J.
BARTON, Ethel S.	HOWORTH, Sir Henry H., K.C.I.E., M.P., F.R.S.	POTTER, Prof. M. C., M.A., F.L.S.
BATHER, F. A., M.A., F.G.S.	HOYLE, W. E., M.A., M.R.C.S., F.R.S.E.	PRESTON, S. Tolver, Ph.D.
BEDDARD, F. E., M.A., F.R.S.	HUME, W. F., D.Sc., A.R.S.M., F.G.S.	PYCRAFT, W. P., M.B.O.U.
BEER, Rudolf.	HURST, C. Herbert, Ph.D.	REID, Clement, F.G.S., F.L.S.
BRUCE, William S.	IRVINE, Robert, F.R.S.E., F.C.S.	RENDLE, A. B., M.A., F.L.S.
BUCKMAN, S. S., F.G.S.	JENNINGS A. Vaughan, F.L.S., F.G.S.	RIDEWOOD, W. G., B.Sc.
CARPENTER, G. H., B.Sc.	JOHNSTON-LAVIS, H. J., M.D., M.R.C.S., F.G.S.	RIDLEY, H. N., M.A., F.L.S.
CHUMLEY, James.	JONES, Prof. T. Rupert, F.R.S.	ROWLEY, F. R.
CLARKE, C. Baron, F.R.S., Pres. L.S.	JUDD, Prof. J. W., C.B. LL.D., F.R.S., V.P.G.S.	SCLATER, Dr. P. L., M.A., F.R.S., F.L.S., F.G.S.
COCKERELL, T. D. A., F.Z.S.	JUKES-BROWNE, A. J., M.A., F.G.S.	SCOTT, A. Ritchie, B.Sc.
COLE, Prof. Grenville A. J., F.G.S.	LANKESTER, Prof. E. Ray, M.A., F.R.S., LL.D.	SEWARD, A. C., M.A., F.G.S.
COSTE, F. H. Perry, B.Sc., F.L.S.	LATTER, O. H., M.A.	SHARP, Dr. D., F.Z.S.
CUNNINGHAM, J. T., M.A.	LING, A. R.	SHARPE, R. Bowdler, LL.D., F.Z.S.
DAVISON, C., M.A., F.G.S.	LUCAS, Frederic A.	SHERBORN, C. Davies, F.G.S., F.Z.S.
DAWSON, Sir J. W., C.M.G.S., F.R.S.	MACHEPSON, Rev. H. A.	SLADEN, W. Percy, V.P.L.S., F.G.S.
DICKSON, H. N., F.R.S.E., F.R.G.S.	MARR, J. E., M.A., F.R.S., Sec. G.S.	SMITH, Annie Lorrain.
DISTANT, W. L.	MARSHALL-HALL, Capt. FGS, F.C.S.	SOLLAS, Prof. W. J., M.A., D.Sc., LL.D., F.R.S.
DOUGLASS, G. Norman.	McINTOSH, Prof. W. C., M.A., LL.D., F.R.S.	SOUTHWELL, Thomas, F.Z.S., M.B.O.U.
EVANS, Maurice S.	MEEK, Alexander.	STEBBING, Rev. T. R. R., M.A., F.L.S.
FIELD, Dr. H. Haviland.	MIERS, H. A., M.A., F.G.S.	THELL, Prof. Hjalmar.
FLOWER, Sir William H., K.C.B., D.C.L., F.R.S., etc.	MITCHELL, P. Chalmers, M.A., F.Z.S.	THOMAS, Oldfield, F.Z.S.
FORBES, Dr. H. O., F.R.G.S.	MORGAN, Prof. C. Lloyd, F.G.S.	TIZARD, Staff-Captain T. H., R.N., F.R.S.
FREAM, Prof. W., LL.D.	MURRAY, G. R. M., F.L.S., F.R.S.E.	TURNER, Prof. Sir William, M.B., LL.D., F.R.S.
GASTANG, W. M.A.	NACHTRIE, Prof. Henry F.	VEJDovsky, Prof. F.
GLADSTONE, Rt. Hon. W. E.	NORMAN, Rev. Canon A. M., M.A., D.C.L., F.R.S., F.L.S., &c.	WAITE, Edgar R., F.L.S.
GREGORY, Dr. J. W., F.G.S., F.Z.S.	OGILEY, J. Douglas.	WILLEY, Arthur, B.Sc.
HADDON, Prof. A. C., M.A., M.R.I.A., F.Z.S.	OLDHAM, R.D., F.G.S.	WOODWARD, A. SMITH, F.L.S., F.G.S., F.Z.S.
HAECKEL, Prof. Ernst, M.D., Ph.D., Hon. F.R.S.E.	PAGE, S.	WOODWARD, B. B., F.R.M.S., F.G.S.
HARMER, S. F., M.A., B.Sc., F.Z.S.	PARSONS, F. G., M.D., F.R.C.S.	WRIGHT, Prof. E. Percival, M.A., M.D., F.L.S., M.R.I.A.
HARTOG, Prof. Marcus.		ZITTEL, Geh. Prof. Karl Alfred von.
HAYCRAFT, Prof. J. Berry, M.D., B.Sc.		
HEADLEY, F. W., M.A., F.Z.S.		
HENSLOW, Rev. George, M.A., F.L.S., F.G.S.		

NATURAL SCIENCE for 1895 has published **63** specially contributed **Articles** in all branches of Zoology, Botany, and Geology, besides the large July Number condensing the Results of the "Challenger" Expedition.

NATURAL SCIENCE for 1895 has published **24** full-page **Plates**, illustrating the above-mentioned Articles.

NATURAL SCIENCE for 1895 has reviewed **100 Books**, and noticed **340** Papers, Pamphlets, and Periodicals.

NATURAL SCIENCE for 1895 has contained **45 Text-figures**.

NATURAL SCIENCE for 1895 has given **Obituary Notices** of **53** men of science, and recorded more briefly the deaths of **77** more.

NATURAL SCIENCE for 1895 has announced **210 Appointments**.

NATURAL SCIENCE for 1895 has given the news of **67 Museums**, and of all the leading Societies and Universities.

These statements can be verified by anyone who will buy the Volumes for 1895, which contain 885 large 8vo pages, and are sold for 12 shillings or \$3.50. Specimen copies, post free, 6d.

Apart from the high character of the contents, as shown by the eminence of the contributors, and as testified to by the Scientific and Public Press of all Countries, the above facts show that NATURAL SCIENCE is the **Cheapest** as well as the **Best** Scientific Monthly.

NATURAL SCIENCE for 1896 hopes to accomplish no less work, and will be sent post free for a subscription of 14 shillings (or \$3.50.) Single numbers One Shilling nett.

LONDON: RAIT, HENDERSON & CO., LTD.,  
 22 ST. ANDREW STREET, HOLBORN CIRCUS.