

## How Good Is Your Glut?

Bob Compton, Microscopy Today

Glutaraldehyde (GA) has proven to be an extraordinarily versatile product. Its many uses range from softening leather and paper to disinfecting and sterilizing hospital instruments. It is used, of course, as a fixative in preparing biological samples for microscopic investigations that most readers of this publication will be familiar with. Even though quality questions relating to GA have been well researched and documented, there is still enough disagreement between industry experts to stimulate an enthusiastic discussion on microscopic list servers.

For example, Bradley Cooke of UC Berkeley recently experienced a perfusion problem. Bradley said, "When I perfuse the animal via the left ventricle, frequently the lungs fill up and the brain is poorly fixed, regardless of which fixative I use. What's perplexing is that it seems to occur only when the fixative is added; typically the blood flush goes perfectly well. I usually don't move the 18 ga. needle when the solutions are changed. Should I be putting the needle in the aorta, as opposed to the left ventricle? How can I do that without damaging the vessel?"

I perfused a couple animals with 1% paraform/1.25% glutaraldehyde and found the brains to be mushy - virtually unsectionable. Despite the problems with the lungs filling up & my worries about fixation, the liver appeared to be hardened, so I assumed something had happened. The glut was opened less than a 1 year ago, but I made the solution 48 hours before the procedure. What might have caused the brain to be so poorly fixed? Should I assume that the perfusion itself was poor, or

does the age and contact with air matter when using glutaraldehyde?"

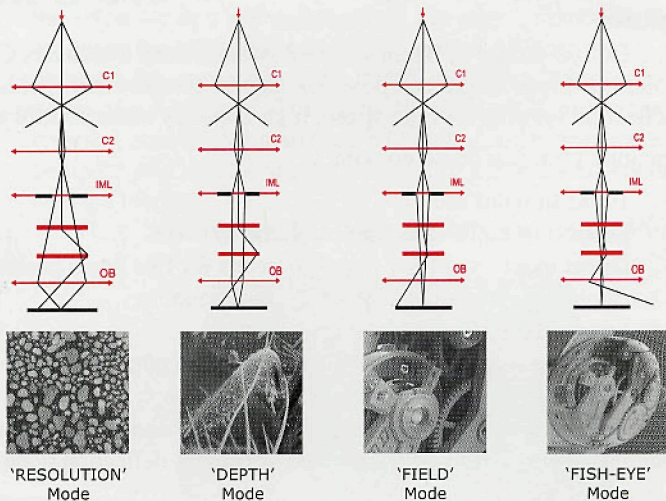
A number of responders had pertinent comments with regard to the perfusion techniques involved, but more interesting were the comments on the GA. For example, Laura Cohen-Gould of Cornell University said, "I haven't done whole animal perfusions in years, so I'll leave comments about that to others, but will say something about the fix solution. Your glutaraldehyde is OLD! It oxidizes over time and becomes pretty worthless as a fixative. My advice is to buy a fresh supply, preferably in small volumes. Many of the EM suppliers sell vials of 10 ml each of a variety of concentrations, sealed under nitrogen or other inert gas, so that you can open a fresh vial and have an easy dilution each time you need to make a fix."

Just to make the "GA is old" point a little clearer, Jim Darley of ProSciTech, states that "GA polymerizes with time and higher temperatures, a nitrogen head does not change much. Frozen, GA will last an awfully long time and if stored refrigerated, most people would still be happy with the product after six months and more. While in the lab, storage should not be a problem although there are other times where we can be sure that inappropriate storage is common. For example, one problem may occur when in transit, as during the summer months. The GA could see some rather high temperatures during a truck or even an air journey. We have always taken some care with GA, but being a long way from the manufacturer (in Australia), we have adopted special precautions such as shipping with ice and mostly by air. Incoming shipments are also packed in ice and refrigerated whenever not in the air. It's costly, but this assures a better product. The high purity Formaldehyde 16% does not require refrigeration, keeps for years and is an

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excellent fixative. I don't know how well this works in perfusion fixation, but it may well be a good alternative."

As Chuck Garber of SPI, a GA vendor notes, "Sometimes even good technical people have honest differences of opinion. I am sure this is one of those instances.

I have always been led to believe that the mechanism of aging of GA is related to the formation first of dimers and trimers of the monomer (it is an autocatalytic reaction). Thus, the way to ensure longest shelf life is to bring the starting purity down to levels (actually lower than I believe most EM users would need) such that one can really delay (but not indefinitely) the onset of the dimer/trimer reaction from starting. By removing the existing dimers and trimers, you remove that which starts the aging process.

When GA is purified to these levels, one can delay the onset of the aging process to such a degree, that some producers of EM gluteraldehyde guarantee their product to be good for one year at room temperature storage. SPI is one of those and, I reconfirmed, that the Ladd gluteraldehyde is in this category as well. Quite possibly, if not probably, there are other vendors making EM GA at that same level of purity.

But with GA being purified to these levels, and with guarantees from the manufacturer for one year at room temperature, to require cold shipment in ice, or even the use of frozen gel packs (which usually are cheaper) in our opinion, is normally not required. Naturally, upon arrival, we do recommend refrigerated storage, just out of general principles, which might be more the result of "dogma" than real reasons. But we regularly ship the ampouled GA to tropical environments, unrefrigerated, and don't have any particular problems. We would discourage shipment to

tropical environments via air freight, since it could end up sitting in customs for days on end but use of the courier services seems to result in no difficulties.

Now for GA being sold in screw top bottles, this would be another story. If the starting purity is good as indicated, then refrigerated shipment should not, in general, be necessary. However, we would strongly recommend the refrigerated storage upon arrival. Indeed we think this should be a requirement. The guarantees for room temperature storage for one year do not extend to the product sold in the screw top bottles, only in the sealed glass ampoules. We don't know about frozen storage, but usually most users want their product in ready-to-use form and don't want to wait for something to thaw out before using.

Again, different people have different opinions about these matters, and reason for these comments is to point out that there are these differences, and that we do not believe one needs to regularly incur the extra costs that would otherwise be necessary for the refrigerated shipment of GA."

John Arnott of Ladd Research (one of three Glut producers, the others being EMS and Polysciences) indicated that "after consulting with Dr. Garber, we are in general agreement on his position concerning shipping and storing of GA. We believe adding cold shipping requirements are overkill, but on the other hand, if any of our customers feel it is necessary we are glad to oblige. We have a long history of producing highly purified GA and in forty some years have never had a problem with our guarantee of one year at room temperature. There are conditions that could merit special packaging and shipping and, as most manufacturers, we

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would be glad to conform to any requests, but we believe it to be an unnecessary expense for most people.

Jim Darley from ProScitech in Australia further contends that it is a different problem when dealing with manufacturers from another continent. For example, he stated, "I do not believe that Chuck's supplier has something better than vacuum distillation available to achieve highest purity. Not that I would want to get into the game of 'mine is purer than yours', since I have no doubt that at production any of the three (to my knowledge) North American 'refiners' produce the very best GA possible and they are comparable. My main point is that:

1. For infusion, and I understand cyto or immunocytochemistry, the purest possible GA should be used. I have read the suggestion that a small amount of polymer may be good during conventional fixation, as this would impede osmotic shock.
2. I certainly do not agree with Chuck's suggestions that GA may be stored at room temperature for a year and then used for EM fixation - regardless of initial purity. GA is not in this regard to be confused with the high purity Formaldehyde (16%), which does not require refrigeration, keeps for years and is an excellent fixative.
3. The point of cold shipping that I made previously was particularly pertinent to our situation were the material needs to travel a long distance. But especially during our summer months (and the USA can be just as hot), I believe that shipping GA with some ice is worth the extra cost."

### Are we forgetting published data?

Stacey Kirsch of Electron Microscopy Sciences enjoyed all of the bantering regarding the facts and fiction on "Glut", but cannot believe that some people are actually arguing about issues that are so well cited in the literature. Kirsch feels that this argument is going to lead to the confusion of many researchers with no basis at all and answers, "The truth on GA is short and simple and I do not care who is making it. The facts remain the same no matter. For anyone who would like to question these results, I remain at your disposal will open my lab notebooks and results of all of the Glutaraldehyde in the industry today and their corresponding results."

There are only three manufacturers of the final distilled product in the US and each and every one of them shows the same tendency towards temperature versus time. Stacey provided the following quote from one of the existing papers on GA characteristics.

"For general information, and, for most of readers, as a kind reminder, we had selected conclusions made public in *Histochemie*, 30, 1972 (!!!), by Gillet, R., *et al.*, from Queen Elizabeth College, London, England: if the absorbance...is plotted against storage temperature, a direct relationship is obtained between storage temperature (above 0° C) and the appearance of spectral "absorbing material".

The authors wrote, "The most important storage criterion was definitely temperature. Those samples stored at -20° C showed virtually no change in...absorbance characteristics, even after eight months storage ...all samples stored at +20° C, however, showed considerable absorbance peaks...the effect of storage at +37° C...was even more pronounced. It appears very speculative and pragmatic to neglect such important facts, or categorize them as 'dogmas'. Certain manufacturers, who did not receive any complaints (yet) when delivering room tempera-

ture stored GA, should consider themselves extremely lucky, and should scrupulously revise their knowledge about EMS grade GA, regardless if sold in ampoules or screw cap bottles."

#### Lab style GA purification

L. R. Melson of Emory University added a voice of experience by stating: "Being an old timer, I have been around since before the advent of vials of GA. In the 70's the Merck index described a method for purifying GA. using activated charcoal. Basically GA begins to degrade to "multimers" when stored at room temperature. The best fixation is achieved with the monomeric form, therefore the purification protocol was designed to remove the more complex forms.

Method: Mix the concentrated liquid glutaraldehyde (25%) with 1g of activated charcoal per 100 ml of liquid. Allow to stand for 30 minutes, then vacuum-filter through a Whatman #1 filter. Repeat three times. Store at 4°C. Repeat once a year.

I have used this method for more than 30 years and consistently obtained very satisfactory fixation. It is messy, but worth the effort."

Jim Darley also reports that he also purified GA by charcoal filtering and even vacuum distillation over 25 years ago. "I'd like to make the following observations on purifying GA. Charcoal filtered GA is not as good the commercial vacuum distilled product. You should store it in a freezer (or even a domestic fridge) if its for more then a few weeks. The triple charcoal filtered product is good enough for EM fixation, if stored well. It's dubious that it is good enough for cytochemistry applications. Charcoal is a recognized carcinogen (interesting that it used to be prescribed for diarrhea). It is also expensive and difficult ship. Several ml of GA are lost in every filtration. And home filtered' GA is very expensive.

Different parameters may apply elsewhere, so in countries where the hourly wage is 50 cents or less, and a drum of GA fell off a truck at a leather tanning or paper towel factory (its used to soften leather and paper) and somebody is making coconut charcoal down the road ... I would be into that and make my own purified GA too. But not in America!"

Merek Malecki of UCSD comments, "As an alternative to charcoal purification, I run distillation, collect the fractions, and then select the monomeric form on the spec."

John Arnott LADD RESEARCH summarizes the manufacturer's positions by saying that, "Based on recent communications, it appears there is a difference of opinion on the shipping of refined Glut among the three major U.S. refiners. All three refiners know their specific product and their techniques and make their decisions on that basis. If the refiner who adds ice feels their product requires it, I have great respect for that decision. They know their product and know the best way to get it to their customer. All data and history indicates Ladd glut does not require ice. If special circumstances arise, we would be glad to add ice. We all act in good faith. If the refiner who adds ice is right and the others are wrong, I will acknowledge and applaud that decision."

#### Conclusion

It appears that the typical researchers should have no problems with their fixative applications if care is taken in purchasing sealed ampoules, proper shipment and temperature-controlled storage of Gluteraldehyde. ■



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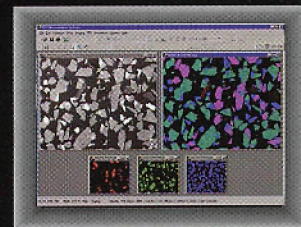
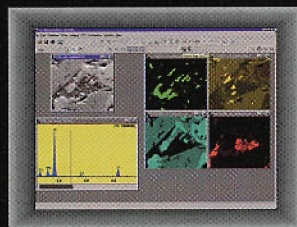
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