How often have you wished that your fatty specimens could be completely infiltrated, that uterine specimens didn't resemble bricks and that your biopsies wouldn't try so hard to be crispy critters? In this note, we would like to explain why these things happen. In the end, we hope that you might get some ideas that will improve your processing to the point that nearly all tissues can be run successfully at the same time.

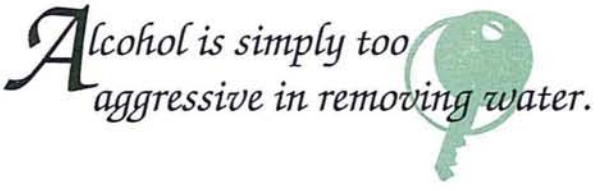
Fixation is rarely the cause of these particular problems, so let's begin with dehydration. Remember that the ultimate goal of processing is to infiltrate the specimen with a wax that is not miscible with the fresh or fixed specimen. Such tissues are filled with 'free' water that simply occupies the spaces among the biological molecules of carbohydrates, proteins, lipids and other compounds. This free water must be removed if wax is to enter, and it is generally done with a graded series of ethanol or isopropanol, followed with a clearant.

There is a second type of water in the specimen, called 'bound' water. Bound water is found surrounding nearly all macromolecules, being held to their surfaces by weak hydrogen bonds. It is not free to move around inside the specimen. Further, it is not necessary to remove it in order to infiltrate the specimen with wax. In fact, the removal of bound

water is precisely what makes bricks and crispy critters. Bound water acts as a spacer among adjacent macromolecules, keeping them from coming close enough together to form hydrogen bonds between themselves. Once this insulating layer of bound water is removed, the tissue shrinks as macromolecules move closer, and the specimen changes its cutting consistency as these molecules bind together. Fibrous organs get tough and specimens comprised mostly of cells become crumbly.

Nearly all compounds (e.g., macromolecules, alcohol, xylene, water, etc.) will engage in hydrogen bonding to some degree or another, although water is the most avid participant. When given a choice between two different molecules, water will hydrogen bond to the molecule with the higher affinity for hydrogen bonding. This basic chemical principle is behind most processing problems.

Alcohol is a very effective remover of water. Being a mobile liquid with low surface tension and low specific gravity, it moves rapidly through most tissues, replacing the free water as it goes. Unfortunately, before finishing that job on the large specimens it begins to strip bound water off the macromolecules of the smaller specimens. The



reason for this is that alcohol is a better hydrogen bonder than most macromolecules. Water readily gives up its hold on the macromolecules and hydrogen bonds instead to the alcohol. Bereft of their insulating layer of bound water, the macromolecules move together and bond to one another.

Alcohol has another problem, albeit much less serious. It will penetrate fat, but it is a rather poor fat solvent. Relatively little fat can be held in solution before the alcohol loses its efficacy in removing water; thus, alcohol must be rotated often.

This brings us to clearing. Because fat and wax are immiscible, all free fat must be removed in order to get complete infiltration. The clearing agent is the major de-fatting solvent in the processing system. Like alcohol in dehydration, the most common clearant (xylene) turns out to a major culprit behind many processing problems.

Aside from the severe health hazards involved with xylene, this clearant is one of the worst for processing a variety of specimens at the same time. Xylene is a powerful solvent that has the capability of further hardening and shrinking the tissues. Increasing the exposure to xylene will help the fatty specimens but only at the expense of the delicate ones. You are thus faced with a dilemma: the changes needed to improve the condition of certain specimens are the very changes that will worsen

others, and you are forced to compromise with a schedule suitable for neither the fatty nor the delicate specimens. To get optimal processing for all types of tissues, the chemistry of the system must be changed. There *are* better dehydrants and clearants available.

If the fault with alcohol lies with it's aggressive hydrogen bonding behavior, why not choose a solvent with a lower affinity for bound water. It sounds easy, and several have been used over the years (dioxane, Cellosolve, S-29 and TissueDry), but these are so toxic that they have no place in your lab. All of them are readily absorbed through the skin and are serious health hazards. In fact, S-29 and TissueDry are no longer commercially available. ANATECH's PRO-SOFT DEHYDRANT, on the other hand, is a blend of solvents that is safe to

PRO-SOFT and PRO-PAR are the gentlest reagents available.

use under normal laboratory conditions, assuming that standard laboratory hygiene and chemical handling practices are followed. Our dehydrant has such low affinity for bound water that it leaves tissues pretty much as they were after fixation, except that free water and some fat have been removed. Shrinkage is much reduced, properly fixed specimens do not get hard and fatty specimens are improved.

PRO-PAR CLEARANT was developed along the same lines. Its affinity for bound water is essentially zero, so tissues can be left in it for long periods without risk of hardening. Because of that, we routinely recommend that three stations of PRO-PAR CLEARANT be used, each for 45-60 minutes, to ensure that all fat is removed from the largest specimens. The third station is inserted in place of one of the alcohol stations.

There are added benefits to using either or both of these products in place of conventional reagents. Besides their safety, they are convenient to use because they reduce the time spent rotating fluids on the processor. PRO-SOFT DEHYDRANT is used full strength, and only one station is discarded at rotation time. Because it is a better lipid solvent, it will last longer than alcohol. If you use an aqueous fixative on your processor, and if the processor is filled nearly to capacity with cassettes, you would discard one station of PRO-SOFT DEHYDRANT only once every 3-5 runs. Lower case loads would allow less frequent rotation. The use of an alcoholic fixative would nearly double the number of runs possible before having to rotate. The more you stretch the rotation period, the more money you will save on solvent and labor costs. We will work closely with you to help determine how far you can go. Most of our PRO-SOFT DEHYDRANT customers are spending less than they were when they used alcohol.

Using three stations of clearant allows you to rotate these fluids less often as well. If you tried this with xylene, however, you probably would overprocess your specimens. Three stations of PRO-PAR CLEARANT will not harm delicate tissues, and will permit less frequent rotation. We suggest stretching the interval between rotations one day at a time until fatty specimens show signs of underprocessing. Your proper rotation time should then be one or two days less than that.

As a final added feature, remember that using less solvent means generating less hazardous waste. In the case of PRO-SOFT DEHYDRANT, you will reduce the volume of waste to one fifth of your

Stretch your rotation schedule, reduce hazardous waste.

current processor-derived alcohol waste *if you do not alter your rotation schedule*. In all likelihood, you will rotate less often and further reduce your waste volume.

If any of this makes technical or economic sense, please give us a call. Describe what you would like to achieve and we will recommend various ways to do it. Solving processing problems is our specialty. Let us help you with yours. 9/95

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