Michael Bray,[1] *M.D.*

The Effect of Chilling, Freezing, and Rewarming on the Postmortem Chemistry of Vitreous Humor

REFERENCE: Bray, M., **"Effect of Chilling, Freezing, and Rewarming on the Postmortem Chemistry of Vitreous Humor,"** *Journal of Forensic Sciences*, JFSCA, Vol. 29, No. 2, April 1984, pp. 404-411.

ABSTRACT: The effect of chilling at the time of death on the postmortem chemistry of the vitreous humor was studied by using sheep heads obtained immediately following decapitation. One group of heads was kept at room temperature, while the remainder were chilled on ice or in ice water, then refrigerated or frozen. Vitreous humor specimens were taken at intervals over a 48-h period. Chilling inhibited the fall in the glucose concentration and the total carbon dioxide content and lessened the increase in lactic acid, compared to the room temperature group. Rapid glycolysis resumed when the heads rewarmed to room temperature starting at 6-h postmortem, but did not resume at later points. The rate of rise of the potassium and magnesium concentrations was also diminished in the chilled eyes. Freezing and thawing caused an abrupt increase in the potassium and magnesium levels, but other solutes were unaffected.

KEYWORDS: pathology and biology, vitreous humor, glycolysis

In a recent paper, Bray et al [*1*] reported the results of studies of vitreous humor chemistry performed on a group of plane crash victims who became immersed in near-freezing water at the time of death. It was observed that the glucose concentrations, while not abnormally elevated, were significantly higher than those measured in a comparison group of cases in which death did not occur in a cold environment. This finding suggested that chilling of the eye at the time of death could inhibit anaerobic glycolysis.

In a follow-up study, vitreous humor chemistry data were reviewed for a large number of cases in which death was known to have occurred outdoors, at all seasons of the year [*2*]. An inverse correlation between the environmental temperature at the time of death and the glucose concentration and carbon dioxide content of the vitreous humor was observed. The potassium concentrations tended to be lower in cold weather deaths, as well. It did not prove possible to use chemical criteria to distinguish between deaths caused by cold exposure and other outdoor deaths in winter.

Both studies provided evidence that the environmental temperature at the time of death could markedly affect vitreous humor chemistry values measured at autopsy. The following experiment was undertaken to test that hypothesis in a controlled fashion.

Materials and Methods

Intact sheep heads were obtained in a slaughterhouse within minutes after death. Vitreous humor was removed immediately by syringe from both eyes of three heads to establish baseline

Received for publication 23 June 1983; accepted for publication 12 July 1983.

[1]Deputy medical examiner, Office of the Chief Medical Examiner, Washington, DC; formerly, chief resident, Department of Pathology, Washington Hospital Center, Washington, DC.

chemical values. These and all specimens were initially placed on ice, then kept frozen until analyzed.

Four experimental groups were studied. One group of heads (room temperature group) was kept in plastic bags at room temperature (20 to 22°C). Vitreous humor was obtained from both eyes of three heads at 1 h. Samples were withdrawn from one eye of three heads at 6 h after death and from the other eye at 12 h. This procedure was repeated with two other groups of three heads at 18 and 24 and at 42 and 48 h.

A second group of heads was plunged into a large volume of ice water immediately following decapitation, and kept in the ice water for 1 h (rapid chilling group). Specimens were drawn from both eyes of three heads at this point. The remainder were packed in ice, and later were drained of water, placed in plastic bags, and refrigerated at 4 to 5°C. A group of six heads was removed from the refrigerator at 6 h and vitreous humor was withdrawn from one eye of each. The heads were then allowed to rewarm to room temperature for 6 h, and the other eyes were sampled. This procedure was repeated with two more groups of six heads at 18 and 42 h.

A third group of heads was packed in ice after decapitation, and later drained of water, placed in plastic bags, and kept refrigerated at 4 to 5°C (refrigerated group). Specimens were taken from groups of three heads, beginning at 6 h, with the same time points and intervals of rewarming as for the rapid chilling group.

The fourth group was also initially packed in ice, but was then placed in a freezer at −18 to −20°C, instead of being refrigerated (frozen group). The eyes had not frozen by 6 h, so specimens were taken from three heads at this point in the same manner as the other groups, and again after 6 h of rewarming. Three heads were removed from the freezer at 17 h, and three more at 41 h postmortem. These were placed in lukewarm water for 1 h to thaw the eyes. Specimens were withdrawn at 18 and 42 h, and again after rewarming. Several specimens from the thawed eyes showed red-brown discoloration consistent with hemolysis, but all samples were retained and analyzed.

The specimens were centrifuged before the chemical study. A standard SMA-6 profile was performed by autoanalyzer. Magnesium concentrations were determined by atomic absorption spectroscopy. Lactic acid was measured enzymatically. Osmolality was determined by freezing point depression. Appropriate commercially prepared standards were run concurrently with each analysis.

Results

Means and ranges of solute concentrations for the specimens obtained immediately postmortem are as follows: sodium 142.6 meq/L (140 to 146), potassium 5.0 meq/L (4.7 to 5.2), chloride 132 meq/L (129 to 135), carbon dioxide content 22.5 meq/L (22.0 to 23.1), magnesium 1.34 meq/L (1.2 to 1.5), glucose 53.7 mg/dL (48 to 60), lactic acid 1.32 meq/L (1.1 to 1.4), urea nitrogen 22.6 mg/dL (16 to 28), and osmolality 229 mOsm/L (224 to 233).

The mean concentrations of those solutes whose levels were significantly affected by chilling are plotted against postmortem interval in Figs. 1 to 5. The dotted lines in these figures indicate periods of rewarming. For the room temperature, refrigerated, and frozen groups, each point represents the mean of three measurements. For the rapid chilling group each point represents six specimens, except for lactic acid, for which only three specimens were analyzed at each time point. The frozen group was not analyzed for lactic acid concentration or osmolality. Results for the various solutes will be summarized individually.

Glucose

The vitreous humor glucose concentration falls rapidly postmortem in the room temperature group (Fig. 1). At 1 h the mean level is 35% below the baseline value, and at 6 h it has fallen by more than 70%. Values near zero are approached by 18 h.

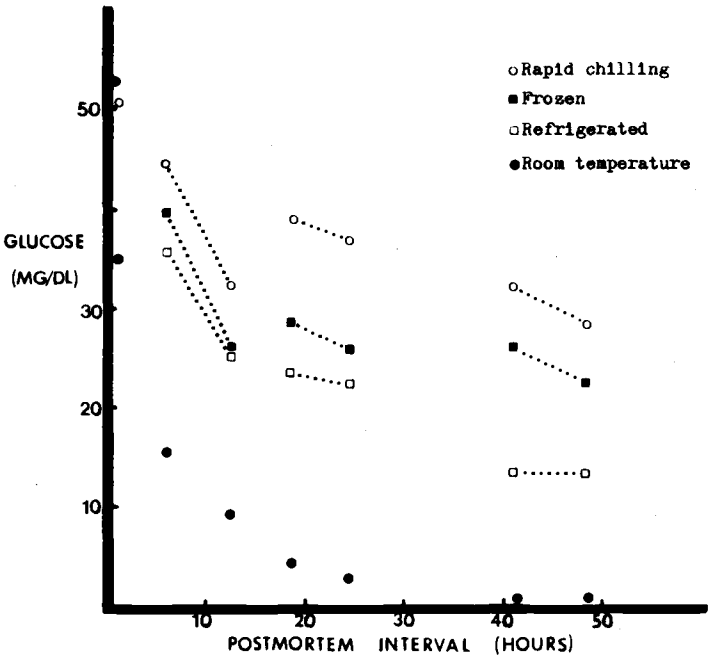

FIG. 1—*The relationship between mean vitreous humor glucose level and postmortem interval for the various groups. Dotted lines indicate periods of rewarming.*

The decline is markedly inhibited in all three chilled groups. Inhibition is most evident in the group subjected to rapid chilling in ice water, for which the mean glucose concentration at 1 h is only slightly below the baseline level. The lowest values are seen in the refrigerated group, which had the slowest rate of cooling. The glucose levels continue to decline through the two-day period.

The interval of rewarming beginning at 6-h postmortem results in an accelerated fall in the glucose concentrations in all three groups. At 18 h and beyond this effect is no longer seen.

Lactic Acid

The influence of chilling on lactic acid levels is the opposite of its effect on glucose concentration (Fig. 2). At room temperature there is an early, rapid rise in lactate, followed by a slower climb, while in the chilled groups only a gradual increase is observed. An abrupt rise in lactic acid concentration takes place during the first rewarming interval, corresponding to the drop in glucose concentration seen at that time. This effect is not observed during later periods of rewarming. The relatively low lactate levels observed in this study may reflect the fact that these animals died suddenly, in their normal state of health.

Carbon Dioxide Content

The overall pattern is similar to that of the glucose concentrations (Fig. 3). The early, rapid decline in total carbon dioxide content seen in the room temperature group is most effectively inhibited by rapid chilling. Nearly the same result is obtained in the frozen and refrigerated

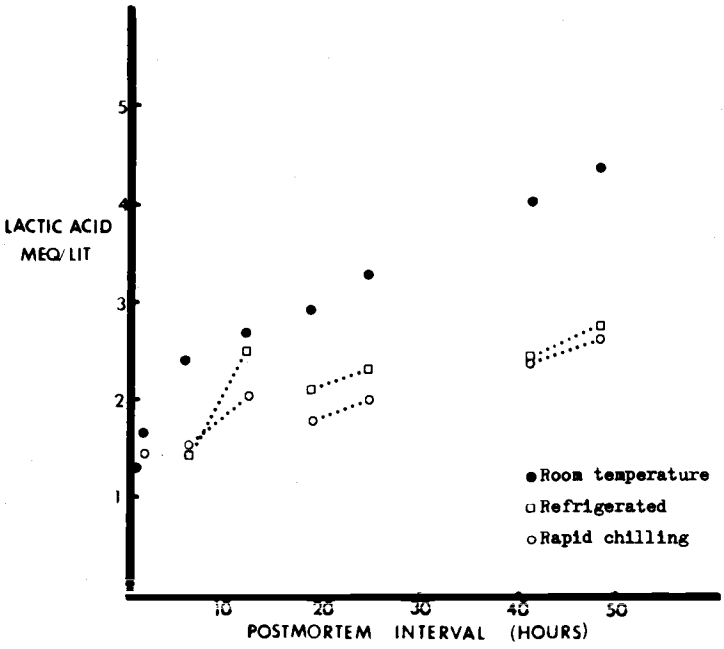

FIG. 2—*The relationship between mean vitreous humor lactic acid concentration and postmortem interval for the various groups.*

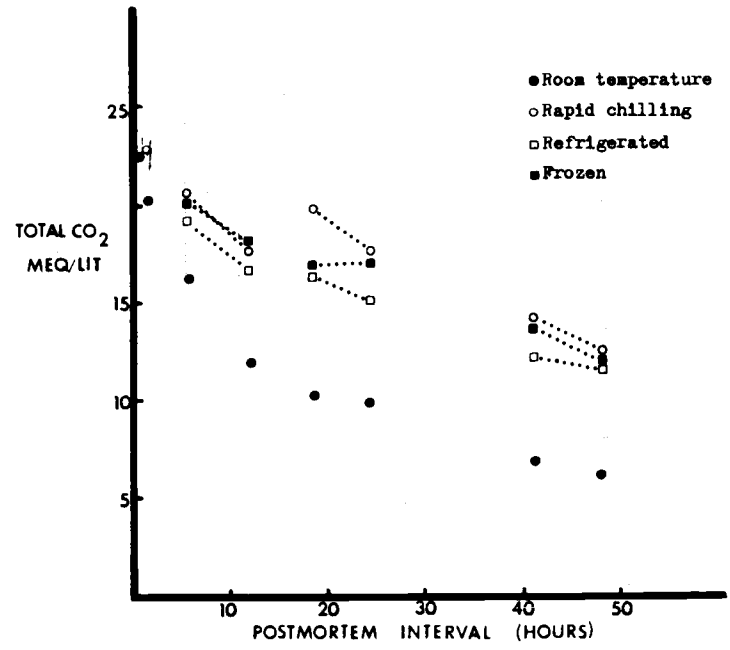

FIG. 3—*The relationship between mean vitreous humor carbon dioxide content and postmortem interval for the various groups.*

groups. In contrast to the glucose results, however, rewarming between 6- and 12-h postmortem does not markedly accelerate the fall in total carbon dioxide content.

Potassium

At room temperature the potassium concentration shows a rapid early rise, followed by a slower, virtually linear increase (Fig. 4). Rapid chilling blunts the early rise and slows the subsequent rate of climb. The same effect is seen, to a lesser extent, in the frozen and refrigerated groups. The rate of rise of the potassium level is not consistently influenced by rewarming. However, thawing of the frozen eyes resulted in an abrupt increase over subsequent periods of rewarming.

Magnesium

The effect of chilling on postmortem magnesium concentrations is similar to that observed for potassium levels (Fig. 5). At room temperature there is an early, rapid rise in magnesium concentration, followed by a steady, near linear climb. The early rise is inhibited by chilling, as is the subsequent rate of increase. Rewarming does not consistently accelerate the rise in magnesium level, but freezing and thawing results in a rapid increase during following rewarming intervals.

Other Solutes

The concentrations of sodium and chloride were similar in all groups during the first 24 h postmortem, but at 42 and 48 h the concentrations of these solutes were lower in the room temperature than in the chilled groups. At 48 h, the mean sodium level of the room temperature

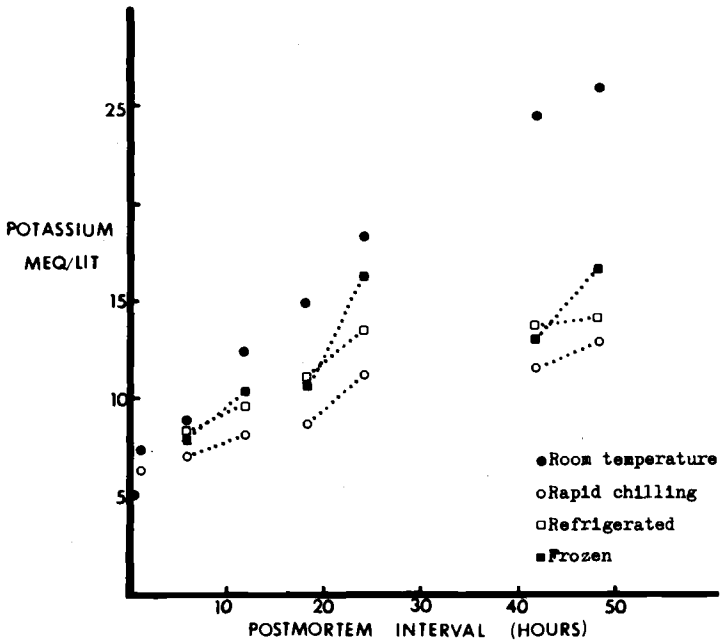

FIG. 4—*The relationship between vitreous humor potassium concentration and postmortem interval for the various groups.*

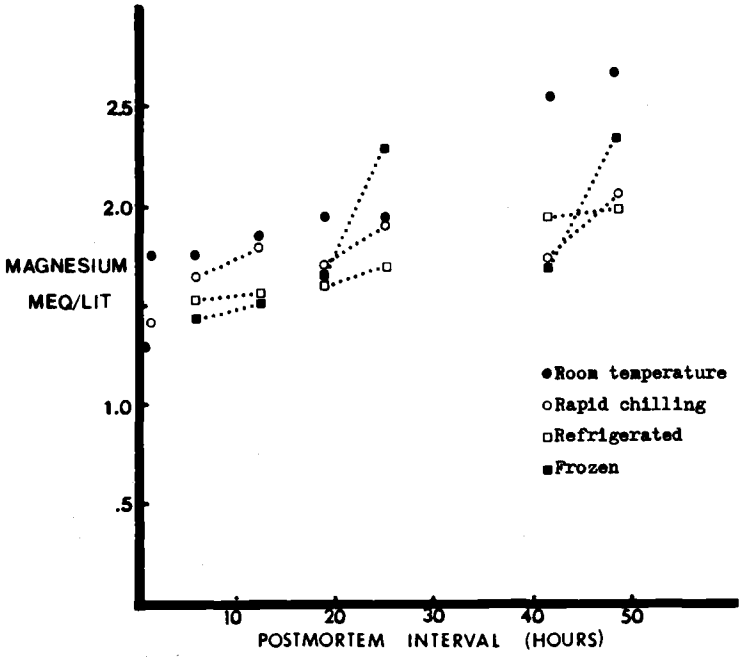

FIG. 5—*The relationship between vitreous humor magnesium concentration and postmortem interval for the various groups.*

group was 120 meq/L, while the means of the chilled groups ranged from 131 to 133 meq/L; the chloride concentration of the room temperature group averaged 112 meq/L, while for the others the mean was 121 to 122 meq/L. Urea nitrogen levels were not significantly affected by temperature or the length of the postmortem interval (data not shown).

Osmolality

Changes in the osmolality of the vitreous humor during the first 24-h postmortem were similar in all groups. There was an early, rapid rise from the baseline mean of 229 mOsm/L. At 6 h the mean value for all groups was 282 mOsm/L, with individual values ranging from 272 to 289 mOsm/L. This rapid increase was followed by a slower climb to a mean for all groups at 24 h of 314 mOsm/L (297 to 320). From that point on, changes were irregular and slight (data not shown). No temperature effect was observed.

Discussion

It is clear from the data that chilling at the time of death can markedly affect the postmortem metabolism of the eye. The consumption of glucose and the production of lactic acid are inhibited in complementary fashion. Diminished acid production results in less consumption of bicarbonate buffer, and the total carbon dioxide content tends to be preserved. In addition to these not unexpected effects on the rate of glycolysis, chilling also inhibits the postmortem rise in potassium and magnesium concentrations, through mechanisms that are not clear.

Other published research in this area is presented in the accompanying paper [*2*]. In summary, studies using enucleated eyes produced results similar to those of the present experi-

ment [3]. However, in two studies employing intact animals, effects of cooling were either minimal or absent [4,5]. Cooling was carried out in air at 4°C; it appears that it was the slow rate of cooling that caused temperature effects on chemistry values to be minimized. The importance of the rate of chilling is demonstrated in the present experiment. The most marked temperature effects were observed in the eyes subjected to rapid chilling in ice water.

The effect of chilling on the rate of rise of the potassium concentration of the vitreous humor clearly makes measurement of the potassium less reliable as a means of estimating the length of the postmortem interval than has been previously supposed [6,7]. For example, the mean potassium concentration at 48 h after death of the eyes subjected to rapid chilling is approximately equal to the mean of the room temperature group at 12 h. Coe has reported that elevated environmental temperatures accelerate the rise in potassium level above that observed at room temperature [8]. Although it might be possible to prepare standard curves of the usual rate of rise of the potassium concentration in various temperature ranges, the actual use of such curves in a practical situation would require the assumption of constant temperature during the postmortem interval.

Another finding also has bearing on the use of the potassium level to estimate the postmortem interval. If the vitreous humor freezes after death, then thaws before autopsy, the potassium level will be markedly elevated. This is presumably caused by lysis of cells in the periphery of the eye, with subsequent diffusion of released potassium ions into the vitreous. The potassium concentration may then appear to indicate a much longer postmortem interval than is actually the case.

Another interesting finding is that the inhibition of glycolysis produced by chilling can be reversed, if rewarming of the eyes takes place during the early postmortem period. Although this phenomenon should be kept in mind when one performs vitreous humor chemistry studies in the investigation of the cause of death of a body found in the cold, it is unlikely to significantly affect chemistry values obtained in the usual autopsy situation, since prolonged rewarming generally does not occur.

Postmortem changes in the osmolality of the vitreous humor have not been previously described. The observed increase in osmolality may result from the cessation of active secretion of intraocular fluids, causing a loss of fluid from the eye, with retention of some solutes. The relationship between postmortem solute concentrations and the rise in osmolality is not clear. This time-dependent change should be kept in mind if one attempts to use the vitreous humor to diagnose a premortem hypoosmolar or hyperosmolar state.

This experiment has documented a number of effects of the environmental temperature on the postmortem chemistry of the vitreous humor. Applications of these findings in human cases are discussed in the accompanying paper [2].

Acknowledgments

The work described in this paper was performed in the clinical laboratory of the Department of Pathology of the Washington Hospital Center. No outside funding was involved. The animal specimens studied were obtained from a federally licensed slaughterhouse, Henry W. Stapf, Inc., of Baltimore, MD (USDA Licence 7402). The cooperation of the firm of Henry W. Stapf, Inc., in this study is greatly appreciated.

References

[1] Bray, M., Luke, J. L., and Blackbourne, B. D., "Vitreous Humor Chemistry in Deaths Associated with Rapid Chilling and Prolonged Freshwater Immersion," *Journal of Forensic Sciences*, Vol. 28, No. 3, July 1983, pp. 588–593.

[2] Bray, M., "The Eye as a Chemical Indicator of Environmental Temperature at the Time of Death," *Journal of Forensic Sciences*, Vol. 29, No. 4, April 1984, pp. 396–403.

[3] Bito, L. Z. and Salvador, E. V., "Intraocular Fluid Dynamics: Postmortem Changes in Solute Concentrations," *Experimental Eye Research*, Vol. 10, No. 2, Oct. 1970, pp. 273-287.
[4] Bannister, D. W., O'Neill, I. E., and Whitehead, C. C., "The Postmortem Diagnosis of Fatal Hypoglycaemia Using the Fatty Liver and Kidney Syndrome of Chicks as a Model," *British Journal of Nutrition*, Vol. 47, No. 2, March 1982, pp. 235-241.
[5] Schoning, P. and Strafuss, A. C., "Postmortem Biochemical Changes in Canine Vitreous Humor," *Journal of Forensic Sciences*, Vol. 25, No. 1, Jan. 1980, pp. 53-59.
[6] Adelson, L., Sunshine, I., Rushforth, N. B., and Mankoff, M., "Vitreous Potassium Concentration as an Indicator of the Postmortem Interval," *Journal of Forensic Sciences*, Vol. 8, No. 4, Oct. 1963, pp. 503-514.
[7] Coe, J. I., "Postmortem Chemistries on Human Vitreous Humor," *American Journal of Clinical Pathology*, Vol. 51, No. 6, June 1969, pp. 741-750.
[8] Coe, J. I., "Some Further Thoughts and Observations on Postmortem Chemistries," *The Forensic Science Gazette*, Vol. 5, No. 5, 1973, pp. 2-6.

Address requests for reprints or additional information to
Michael Bray, M.D.
Deputy Medical Examiner
Office of the Chief Medical Examiner
19th & Massachusetts Ave., SE
Washington, DC 20003