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Cardiac Blood pH as a Possible Indicator of Postmortem Interval

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ABSTRACT: Postmortem changes in the pH of blood and selected tissues in rats were evaluated at intervals ranging from 2 min to 96 h. Cardiac blood pH was significantly and reproducibly decreased in all groups at all postmortem intervals, independent of the method of sacrifice used. A preliminary study using cardiac blood obtained at autopsy from a limited number ($n = 11$) of human subjects demonstrated a significant negative correlation ($r = -0.908$, $P < 0.01$) between postmortem interval (range 2 to 20 h) and cardiac blood pH.

KEYWORDS: pathology and biology, postmortem interval, postmortem examinations, pH changes, thanatology, time of death, acidosis

Among the challenges faced by the forensic science investigator is the estimation of the postmortem interval in cases in which the time of death is uncertain or even completely unknown. The accuracy of such estimates may range from within a few hours, in the case of a recent death, to weeks or even months, when only decomposed or fragmentary remains are available. This study is concerned with the evaluation of relatively short postmortem intervals of four days or less.

Physical evidence such as body temperature, rigor mortis, and livores have long been used to provide a rough estimate of the postmortem interval. Other methods such as induction of supra-vital reactions upon stimulus, postmortem pupillary reactions following homatropine, and postmortem excitability of the sweat glands have also been reported [*1*]. More recently, a variety of procedures have been described based on changes in biochemical parameters after death. Nonprotein nitrogen in cisternal fluid [*2*] and potassium levels in the cerebrospinal fluid [*3*] and intraocular fluid [*4*] have been shown to correlate with postmortem interval. In general, however, these methods provide equivocal results. In the case of potassium levels in the vitreous humor, the variability can be ± 20 h over a range of 0 to 120 h [*5*]. Most recently, 3-methoxytyramine in the putamen of the brain has been shown to increase after death in a highly reproducible manner [*6*]. The results, however, may be distorted in subjects who have suffered from organic heart disease.

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Hydrogen ion concentration in tissues and body fluids is known to increase after death [7-9]. This phenomenon was discovered many years ago. Early studies, which attempted to correlate tissue or body fluid pH with a postmortem interval, were reported to have failed "because of the wide range of variations which have been observed for any given post-mortem time" [1]. These early studies were conducted before the advent of electrometric techniques of measuring blood pH. Since the introduction of the pH meter, several factors relevant to accurate blood pH determinations have also been identified [10]. Blood pH, in vitro, has been shown to depend on storage, temperature, and intrinsic metabolic activity. The pH of stored blood is known to decrease unless a metabolic inhibitor, for example, 0.06% sodium fluoride, is added. Further, the importance of drawing blood specimens anaerobically has been demonstrated [8].

In a single study which used an electronic pH meter, the pH of postmortem blood was evaluated as a possible indicator of antemortem acid-base status [8]. The pH of blood from various sites was determined up to 9 h postmortem using ten acidotic patients with documented antemortem metabolic or respiratory acidosis and six control patients. The pH of left ventricle cardiac blood was found to decrease in proportion to the length of time after death. A 0.2 pH unit difference between normal and acidotic patients was detected.

No further studies evaluating cardiac blood pH as an indicator of postmortem interval have been reported since 1957. In a related study, however, a highly reproducible decrease in pH, at intervals up to 96 h postmortem in rat cardiac blood and tissues, has been observed and shown to correlate with the postmortem redistribution of morphine at each site [11]. For the purpose of evaluating pH as a possible indicator of postmortem interval and its possible involvement with postmortem drug redistribution, we have conducted a series of pH studies, at controlled postmortem intervals, in rats, and we have also studied a small number of human cadavers for which an accurate postmortem interval was available.

Materials and Methods

pH Measurement

Hydrogen ion concentrations were determined using a Corning pH meter, Model 10 with a Corning No. 476223 Calomel combination electrode (Corning Glass Works, Corning, New York). Certified calibration buffer standards (Fisher Scientific, Fair Lawn, New Jersey) were used before each pH analysis at 21°C.

Animal Studies

Groups of four male, Sprague-Dawley rats weighing 190 to 387 g were allowed food and water ad libitum before blood sampling by orbital sinus puncture. Immediately after sampling, the rats were killed by cervical dislocation or carbon dioxide asphyxiation. Some groups were necropsied after 2 min, while others were maintained intact at 21°C up to 96 h before necropsy and pH measurement. Specimens of orbital sinus blood or cardiac blood or both obtained at necropsy were immediately measured for pH by direct immersion of the pH electrode in the whole blood. All pH analyses were accomplished at 21°C within 5 min of necropsy.

Human Studies

Eleven random deceased, human subjects, for whom the time of death was accurately known, were autopsied 2 to 20 h postmortem. At the time of autopsy, specimens of left heart blood were drawn using a syringe, taking care not to introduce room air into the specimen. The specimens were then transferred to sealed, additive-free Vacutainer® blood collection

tubes by insertion of the syringe needle through the rubber stopper. Specimens were stored at 5°C and rewarmed to 21°C before pH determination on the same day. Age, sex, postmortem interval, cause of death, and cardiac blood pH were recorded. The relationship between postmortem interval and cardiac blood pH was evaluated by regression analysis.

Results and Discussion

Blood pH studies carried out on rats killed by carbon dioxide asphyxiation revealed a significant decrease in blood pH from premortem values of 7.34 ± 0.02 (mean ± standard error) to 6.74 ± 0.05 5 min after death and to 5.74 ± 0.05 and 5.55 ± 0.03 after 48 and 96 h, respectively (Fig. 1). The observed rapid decrease in cardiac blood pH was apparent in rats killed by cervical dislocation as well as in those asphyxiated with carbon dioxide, suggesting that the acidosis was not due to the carbon dioxide inhalation. This rapid decrease in cardiac blood pH supports the observation by Jetter [7] that the greatest change in pH occurs during the early postmortem interval.

Although there was no significant difference in postmortem pH associated with the method of sacrifice, the groups of rats sacrificed by carbon dioxide asphyxiation showed less variability in cardiac blood pH at any given sacrifice time compared with animals killed by cervical dislocation (Fig. 1). This may be related to cardiovascular injury and shock, since considerable hemorrhage and pooling of blood was revealed upon necropsy of the cervically dislocated animals. Animals killed by carbon dioxide asphyxiation showed declining cardiac blood pH with relatively low variability throughout the studied postmortem interval.

Several mechanisms may be involved in producing these observed pH changes. The sharp decrease in blood pH immediately after death is consistent with reports from previous animal and human studies [7, 12]. These investigators have shown that carbon dioxide accumulates until available oxygen is exhausted [12], resulting in the initial pH decline. Thereafter, there is a gradual decline in pH as the result of postmortem metabolism of glucose with resulting lactic acid and phosphoric acid accumulation [8]. Kastenschmidt et al. [13] have demonstrated that fast-glycolysing muscles may show a pH of 5.5 or less only 30 min after death as a result of postmortem glycolysis. Hydrogen ion movement out of muscle tissue into

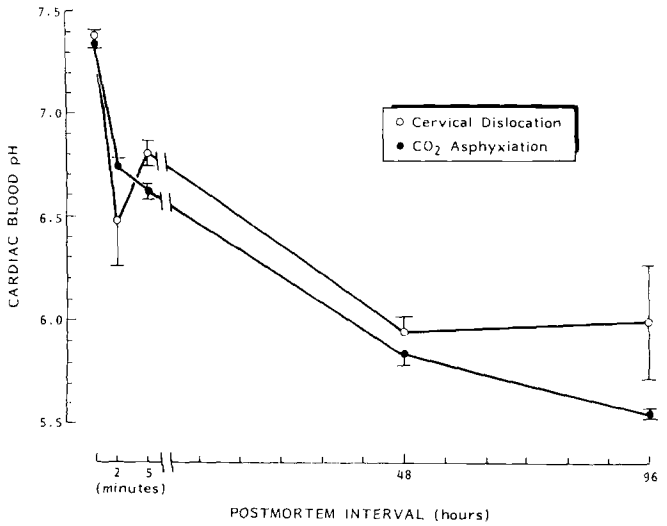

FIG. 1—*Effects of postmortem interval on cardiac blood pH in rats.*

blood may, therefore, account for the continued reduction in cardiac blood pH to 96 h postmortem. Note also that the decline in pH does not continue indefinitely. It has been reported [*1*] that, for human remains, the pH will begin to rise again after approximately four days (the limit of our current study).

Human Studies

A limited number of human cases was evaluated to determine if the results from the above studies of cardiac blood pH in rats could be extended to the evaluation of human remains. For the eleven cases studied, a strong negative correlation ($r = -0.908$, $P < 0.01$) was found between postmortem interval and cardiac blood pH (Fig. 2). From the regression analysis relating these two parameters, the estimated postmortem interval was derived for each case and compared with the known value (Table 1). The mean absolute error in the estimated postmortem interval is 2.1 ± 0.5 h and appears to be relatively consistent over the 20-h period considered. Such a pattern of variability results in a greater relative error (percent overestimation or underestimation) for a short postmortem interval. It appears, however, that these results are considerably less variable than those reported in the previously cited literature. Clearly, a more extensive study will be required, using a larger number of human subjects, to establish the full range of variability and the effects of other intrinsic and extrinsic factors, including preexisting disease states, cause of death, and temperature of the postmortem environment. In particular, the effects of conditions which may lead to a lactic acidosis or other metabolic acidosis prior to death (for example, uncontrolled diabetes mellitus) will require thorough evaluation.

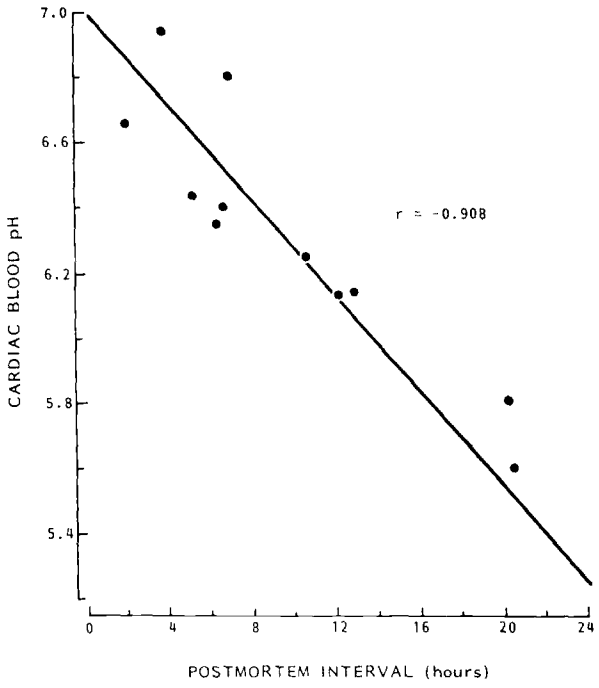

FIG. 2—*Effects of postmortem interval on cardiac blood pH in humans.*

TABLE 1—*Postmortem interval (PMI) and cardiac blood pH from coroner's cases.*

Age, years	Sex	Cause of Death	Cardiac Blood pH	Actual[a] PMI, h	Estimated[b] PMI, h	Error, h
...	...	...	6.64	2.0	4.3	+2.3
58	M	cancer	6.95	3.7	0.0	−3.7
...	...	...	6.43	5.3	7.9	+2.6
35	M	asthma	6.35	6.5	9.3	+2.8
30	M	accident[c]	6.40	6.8	8.4	+1.6
...	...	...	6.81	6.9	1.4	−5.3
51	M	alcoholism	6.25	10.6	11.0	+0.4
79	F	CHF[d]	6.15	12.0	12.7	+0.7
45	F	choking	6.15	12.8	12.7	−0.1
0.2	M	SIDS[e]	5.81	20.3	18.5	−1.8
...	...	...	5.60	20.4	22.1	+1.7

[a]Documented postmortem interval.
[b]Estimated postmortem interval from regression analysis.
[c]Auto accident, severely burned, blood ethanol 187 mg/dL.
[d]CHF = congestive heart failure.
[e]SIDS = sudden infant death syndrome.

In summary, controlled studies in rats have demonstrated reproducible, postmortem changes in the pH of cardiac blood. A pilot study in human subjects suggests that such changes in cardiac blood pH may provide a quick and convenient method for estimation of the postmortem interval to supplement existing techniques.

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