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Lactic Acid Concentrations in Vitreous Humor: Their Use in Asphyxial Deaths in Children

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ABSTRACT: Lactic acid concentrations in brain tissue of humans have been shown to increase with an extended agonal period. Infants and children dying from various causes are undergoing different stress conditions terminally and the postulate of this study is that natural death cases and traumatic asphyxia cases are characterized by varying agonal periods, the former being somewhat prolonged with the latter being rather brief. One-hundred-and-two cases of infants and children were examined for vitreous humor lactic acid concentrations. They were divided into two major categories, Sudden Infant Death Syndrome (SIDS) and non-SIDS cases. SIDS was further divided into SIDS without additional findings and SIDS with secondary findings which contributed to death. The non-SIDS category included traumatic asphyxia cases as well as those dying from blunt trauma, known respiratory diseases, and other causes. Categorical mean values and standard deviations were calculated. The vitreous humor lactic acid mean value for traumatic asphyxia was significantly lower than the mean value for SIDS. Also the mean value for known respiratory diseases was statistically lower than the mean value for SIDS with secondary findings. These findings are probably suggestive of agonal time differences and may be a reflection of the various mechanisms of death.

KEYWORDS: pathology and biology, asphyxia, vitreous humor, lactic acid, sudden infant death syndrome (SIDS)

The value of postmortem chemistry determinations in a variety of situations has been well demonstrated by Coe [*1*]. In many cases, the results have been helpful in ascertaining the nature of the biochemical abnormalities that result in death. In other instances, these findings have established the cause of death in situations in which significant pathological findings have not been forthcoming as in the absence of a complete autopsy or some other complicating circumstances, and use of the vitreous body as the medium for those analyses has been shown to be fruitful [*2*]. The globe of the eye, of which the vitreous is the major compo-

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nent, is anatomically well protected and resists putrefaction to a greater extent than the other body fluids. For these reasons, it is especially attractive to forensic pathologists as the medium for which certain quantitative measurements can be made despite a lengthy postmortem interval (PMI).

The use of lactic acid levels in the vitreous body appears to have potential for elucidating the metabolic circumstances, especially those accompanied by anaerobic glycolysis associated with the agonal period before death, since these various conditions have a well established biochemical basis [*3*]. A knowledge, therefore, of the concentration patterns of lactic acid as an end product of glycolysis can be most informative provided that the routes of metabolism are known.

It has been demonstrated by Townsend that in aircraft accident victims, lactic acid levels over 200 mg/dL in the central nervous system (brain) tissue are indicative of hypoxia [*4*]. It is therefore believed that a variety of conditions, such as lack of oxygen from shock, drowning, drugs, and so forth would also produce an elevated lactic acid value. Ocular tissue (vitreous) has been studied by Cole [*5*] and Cohen and Noell [*6*], and the routes of metabolism in this tissue appear to be well documented.

There exists a sequence of chemical events in the body as a whole, and the ocular portion in particular, which takes place at the time of death, and may or may not continue after death, leading to the accumulation of lactic acid in the vitreous humor. The hypothesis of the present study is that a prolonged agonal period before the moment of death would result in an elevated lactic acid in the blood and the vitreous, and that such an increase would reflect the duration and severity of such exertion or stress. Given a normal value for nonstressful death, the postmortem change in the concentration of lactic acid can be followed since, as Graymore proposed, it appears that the increase does not arise from glycolysis of the endogenous vitreous glucose [*7*].

Materials and Methods

Lactic acid determinations were performed in those cases in which there were sufficient specimens available after completion of the routine laboratory tests. All subjects were from the Medical Examiner's Office of Rhode Island and were consecutive over a period of several years. All bodies were taken to the morgue after death, or as soon after discovery as possible, and maintained there at a temperature of 4°C. In many cases, the time of death was accurately known, but very often it was not; the vitreous potassium levels were used to approximate the PMI in the latter cases.

In each case, the vitreous humor from both eyes was completely aspirated from the posterior chamber of the lateral angle. A 12-cc disposable plastic hypodermic syringe with attached No. 20 gauge needle was used and the receptacle was a rubber-stoppered, chemically cleaned glass test tube. All of the specimens were transported to the in-house laboratory and stored at the same temperature until the time of analysis. Those specimens appearing very cloudy or discolored were not included in these experiments.

The enzymatic method of Hohorst [*8*] was adapted for vitreous lactic acid determinations. The chemical synopsis of this procedure is detailed in Fig. 1. Perchloric acid was the reagent of choice in making the protein-free filtrates. Because of the high concentration of lactic acid in the specimens, dilution was necessary to bring them into optimal analytical range; this was accomplished after the filtrate had been prepared. The increase in absorbance was measured using a Beckman DU spectrophotometer. The procedure of Segal et al [*9*] was modified to accommodate vitreous pyruvic acid determinations. Perchloric acid was used in making protein-free filtrates and also the readings were made on the Beckman DU spectrophotometer.

The routine determinations including glucose, urea nitrogen, chloride, sodium, and potassium were accomplished using classical methods, the first two being done on a

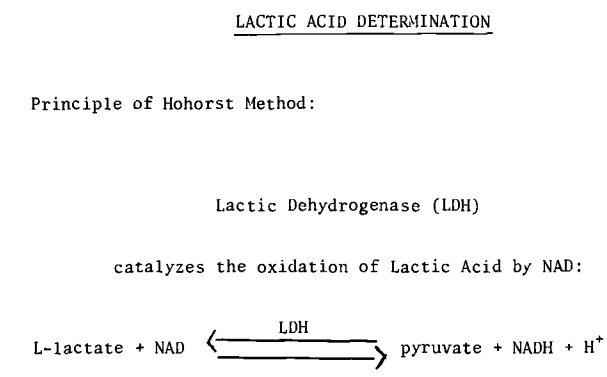

FIG. 1—*Chemical synopsis of enzymatic method of Hohorst adapted for vitreous lactic acid determinations.*

Technicon Dual Channel Autoanalyzer. A Technicon Single Channel Autoanalyzer was set up to accommodate chloride analyses and the metal concentrations were measured with atomic absorption spectrophotometric techniques on a Perkin-Elmer Model 303 instrument. Whenever deemed necessary, creatinine levels were obtained using alkaline picrate without Lloyd's reagent.

In many cases investigated, vitreous total protein determinations were made. Whenever possible, two methods were used, both to assess the accuracy of quantitation and to attempt the ascertainment of the composition profile. The colorimetric technique modified for this purpose was of the biuret method while the turbidimetric method involved the use of the trichloracetic acid. For comparative studies, pooled specimens were dialyzed against 2*N* sodium chloride solutions and then assayed for protein content according to the two methods.

To document the stability of some of the constituents of the vitreous humor under the storage conditions, additional experiments were undertaken. In many cases glucose determinations were made as soon as possible after procurement of the specimens and these were compared to the values found after specimens had been stored under normal laboratory conditions for extended periods of time, in some instances, up to two weeks. The inherent instability of lactic acid and pyruvic acid in biological fluids has long been accepted, but whether or not this is true in the vitreous humor has not been established. To this end, vitreous from a number of subjects, immediately upon aspiration, was proportioned three ways: one according to normal protocol, the second into a tube containing the preservatives (sodium fluoride and sodium oxalate), and the third, in a fixed volume, was added to a predetermined quantity of perchloric acid. Glucose, lactic acid, and pyruvic acid tests were performed on each specimen.

Results

In vitreous humor, the mutual concordance of the test points measuring glucose, lactic acid, and pyruvic acid established experimentally the stability of lactic acid in this medium. This set of experimental evidence bears out the importance of vitreous humor as a stable and independent medium.

Although von Heyningen lists the normal concentration of vitreous lactic acid as 70 mg/dL [*10*], in 40 vitreous humor specimens from young healthy adults who died in a "relaxed" disposition, values ranging from 74 to 153 mg/dL and a mean lactic acid level of 120 $\pm$ 30 mg/dL was calculated. These experimental results are shown in Table 1. These cases

TABLE 1—*Lactic acid concentrations in young healthy adults who had "instantaneous deaths."*

Medical Examiner No.	Lactic Acid, mg/dL	Cause of Death[a]
2175-75	135	GSW
2217-75	148	GSW
2223-75	123	GSW
2257-75	144	GSW
2284-75	136	MV Acc.
2299-75	101	CCT
2292-75	83	CCT
2341-75	129	M.I.
2356-75	110	CCT
0185-76	119	GSW
0454-76	139	MV Acc.
0414-76	137	MV Acc.
0564-76	129	GSW
0700-76	139	MV Acc.
0801-76	89	GSW
1276-76	93	MV Acc.
1339-76	130	MV Acc.
1405-76	74	MV Acc.
1635-76	90	MV Acc.
1737-76	128	MV Acc.
1939-76	100	MV Acc.
2398-76	149	MV Acc.
2825-76	120	MV Acc.
3111-76	80	MV Acc.
0632-77	103	GSW
1545-77	145	CCT
1678-77	148	GSW
1948-77	128	MV Acc.
1949-77	112	MV Acc.
1974-77	128	MV Acc.
2023-77	93	MV Acc.
2052-77	130	GSW
2256-77	111	GSW
2262-77	115	MV Acc.
2309-77	116	MV Acc.
2374-77	153	MV Acc.
2375-77	133	MV Acc.
2474-77	122	GSW
2682-77	125	CCT
2686-77	97	CCT

[a]GSW = gunshot wound, MV Acc. = motor vehicle accident, and CCT = craniocerebral trauma.

represent healthy adults under 35 years who received no medical attention at the time of their deaths, and whose deaths were determined to be instantaneous.

One-hundred-and-two cases of infants and children were examined for vitreous lactic acid concentrations. Tables 2 and 3 reveal categories of Sudden Infant Death Syndrome (SIDS) cases with no additional findings and with additional findings, respectively. SIDS cases also had petechiae present consistently, with the exception of a single case. The range of values for SIDS without secondary findings is 86 to 310 mg/dL and the range for SIDS with secondary findings (that is, pathology noted but insufficient to cause death), is 122 to 485 mg/dL. Table 4 reveals cases of traumatic asphyxia including the specific circumstances responsible for demise. Minimal or no petechiae were observed in this group of cases. The range of lactic

TABLE 2—*Lactic acid concentrations in infants—SIDS cases with no secondary findings.*[a]

Number	Age	Lactic Acid, mg/dL	Glucose, mg dL
2279-75	2 months	220	52
2964-75	4 months	144	25
0892-76	2 months	167	16
1009-76	3 months	144	76
1101-76	3 months	138	166
1402-76	7 months	86	<5
2001-76	8 months	171	40
2264-76	3 months	186	25
3150-76	4 months	220	132
0429-76	6 months	186	42
0452-77	3 months	153	30
0495-77	2½ months	125	24
2533-77	6 months	142	24
2695-77	3 months	212	27
3227-77	15 days	239	24
3153-77	3½ months	252	QNS[b]
0270-78	4 months	201	45
2159-79	3 weeks	164	30
2845-79	9 weeks	98	30
0111-80	3 months	190	78
0004-80	1 month	264	16
0420-80	1 month	112	38
1028-80	8 days	305	32
2880-80	7 months	270	30
2961-80	2 months	310	56
0743-81	2½ weeks	170	<5

[a]Petechiae present in all cases except one.
[b]QNS = quantity not sufficient.

TABLE 3—*Lactic acid concentrations in infants—SIDS cases with secondary findings.*[a]

Number	Age	Lactic Acid, mg/dL	Glucose, mg/dL
2905-75	3 months	198	130
2283-75	4 months	142	80
0429-76	6 months	186	42
1287-76	5 months	226	<5
1800-76	7 weeks	238	50
3169-76	8 weeks	264	26
0122-77	1½ month	274	44
0250-77	1 month	148	68
0463-77	3 months	149	36
0481-77	1 year	190	82
2644-77	3 months	316	QNS[b]
3237-77	3 months	209	24
3426-77	2 months	122	30
2055-78	5 weeks	242	114
1341-80	7 weeks	485	174
2208-80	3 months	150	28

[a]Few, minimal, or no petechiae present.
[b]QNS = quantity not sufficient.

acid values for these cases is 47 to 234 mg/dL. Table 5 shows a list of unrelated cases with known causes of death other than those categorized in the previous tables. Few or no petechiae were noted in these deaths. The 102 total cases are represented as they relate to each group and each other in Table 6. As can be seen, the mean value for lactic acid in traumatic asphyxia cases is lower than the mean value for SIDS. These mean values are 137 ± 51 and 204 ± 50 mg/dL, respectively. It was also determined that the mean value for known respiratory diseases is lower than the mean value for SIDS. This value for respiratory diseases is 156 ± 30 mg/dL.

Discussion

If one postulates that the Sudden Infant Death Syndrome is characterized by a short agonal period, the systemic lactate would be expected to be normal. If this level is reflected in the vitreous humor, then the level for SIDS victims should be similar to those for other infants dying without a prolonged agonal period. If, on the other hand, the infant undergoes an agonal period of some duration, the lactic acid level might be elevated and this could possibly be detected in the vitreous humor.

Since many traumatic asphyxial deaths are sudden, suggested historically as well by the lack of petechiae [*11*], it may be assumed that lactate levels will be lower in this group of cases than other relatively nonstressful deaths. This has been borne out by the findings of this study. It is further suggested that some respiratory disease fatalities may have a more rapid terminal demise than other unrelated cases.

Since the diagnosis of traumatic asphyxia may be difficult because of incompletely known or unreliable circumstances and lack of overt pathologic findings, the use of lactic acid determinations in vitreous humor, coupled with an assessment of petechiae in the thoracic organs, may be additionally helpful and perhaps suggestive of a death caused by traumatic asphyxia, especially if the historical and scene investigation evidence is corroborative and supportive. Dominguez et al [*12*] reached similar conclusions when interpreting lactic acid values in brain tissue of aircraft accident cases.

Glucose determinations performed concurrently on each of the cases, as shown in Tables 2

TABLE 4—*Lactic acid concentrations in infants—traumatic asphyxia cases.*[a]

Number	Age	Lactic Acid, mg/dL	Glucose, mg/dL	Cause of Death
1553-76	3 weeks	234	41	wedging; compression of airway
0388-77	24 months	158	35	house fire; carbon monoxide poisoning
0810-77	6 weeks	225	24	steam inhalation; ruptured valve
1348-77	24 months	47	76	house fire; smoke inhalation
1504-77	3½ weeks	122	26	suffocation
1562-77	24 months	157	50	drowning; floating in pool
1612-77	6 months	80	8	wedging; found at foot of bed
2333-77	9 months	184	10	wedging
3224-77	7 months	143	25	fire victim; smoke inhalation
2169-78	24 months	150	36	fire victim; smoke inhalation
3005-78	1 month	166	25	vomitus; choked on bolus
0052-79	6 months	104	32	house fire; smoke inhalation
1140-79	22 months	93	54	drowning
1621-79	21 months	86	30	drowning
2506-79	24 months	112	38	carbon monoxide poisoning; smoke inhalation
1513-80	18 months	126	30	drowning

[a]Few, minimal, or no petechiae present.

TABLE 5—*Lactic acid concentrations in infants—other causes of death.*[a]

Number	Age	Lactic Acid, mg/100 mg	Glucose, mg/100 mg	Cause of Death
2346-75	2 months	425	80	electrolyte imbalance; viral enteritis
2615-75	4 months	188	9	maternal heroin addiction with methadone treatment
2283-75	4 months	142	60	unexpected infant death with fatty change of liver; treated one week ago for a cold; family history of diabetes
0146-76	1 year	167	28	congenital hydrocephalus
0458-77	4 months	102	26	Werdnig-Hoffman disease
1540-77	5½ weeks	230	64	Ellis van Greveldt Syndrome; congenital heart disease
2519-77	3 months	213	40	multiple congenital anomalies
0369-78	4½ months	319	24	hemophilus influenza; meningitis, bronchopulmonary dysplasia
1022-78	7½ months	178	52	electrolyte imbalance; secondary d/t gastroenteritis of diarrhea and dehydration
2800-79	3½ months	71	28	Marked diffuse fatty liver; hereditary storage disease; probably uric acid cycle def. disease
1461-79	10 months	172	34	meningococcal septicemia
2186-79	6 months	80	26	Became apneic and cyanotic in bed; Acute purulent meningitis
2752-79	3 months	116	QNS[b]	acute encephalitis
3180-79	2 months	315	96	unknown etiology; acute electrolyte imbalance
0602-79	2 days	182	264	multiple congenital anomal and prematurity; secondary s/p gastrostomy and ligation of a tracheosophageal fistula
1736-80	3½ months	226	QNS[b]	electrolyte imbalance; unknown etiology

[a]Few, minimal, or no petechiae present.
[b]QNS = quantity not sufficient.

through 6 show that no correlation exists with lactic acid values and that the latter are independent in formation and variability. Exogenous glucose administered in terminal therapeutic situations, and stress-produced gluconeogenesis from glycogen in the liver probably accounts for several isolated cases of hyperglycemia without concurrent elevations in lactic acid.

Conclusion

Although there appears to be a wide range of lactic acid levels as a result of this study, the mean values, assessed with history, circumstances, and all autopsy findings, especially the appearance of petechiae or lack thereof, might be useful in distinguishing traumatic asphyxia cases from true SIDS cases. Therefore, decreased lactic acid concentrations in vitreous humor appear to be an additional diagnostic marker in cases of infant deaths from traumatic asphyxia.

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TABLE 6—*Lactic acid concentrations in infants—total cases.*

Cause of Death	Number of Cases	Lactic Acid in mg/dL Range	Lactic Acid in mg/dL Mean Value	Significance[a]	Glucose in mg/dL Mean Value
All SIDS	42	...	204 ± 50	$t = 2\,r = 0.951$, $P < 0.05$	53 ± 17
SIDS	26	86-310	187 ± 48	$t = 2\,r = 0.203$, $P < 0.05$	43 ± 9
SIDS with secondary findings	16	122-485	221 ± 52	$t = 3\,r = 0.175$, $P < 0.05$ $t = 2\,r = 0.480$, $P < 0.05$[b]	62 ± 22
Total non-SIDS	60	...	168 ± 53	...	44 ± 19
Trauma	15	...	187 ± 34	...	60 ± 45
Asphyxia, traumatic	16	47-234	137 ± 51	...	31 ± 13
Respiratory disease	13	...	156 ± 30	...	28 ± 6
Other causes	16	71-425	195 ± 83	...	59 ± 20
Non-SIDS without trauma	45	...	163 ± 56	...	39 ± 29
Total causes	102	...	186 ± 53	...	49 ± 25

[a]Significance represents the statistical difference between the mean value of traumatic asphyxia cases and each category; t = student's t test; r = correlation coefficient; and P = probability.

[b]The statistical difference between SIDS with secondary findings and respiratory disease.

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