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Use of Chemical Determinations on Vitreous Humor in Forensic Pathology

It is frequently important in deciding the cause of death to be able to determine chemical abnormalities which may have existed prior to demise. This is particularly true in cases where clinical history suggests the possibility of such chemical abnormalities, but opportunity for autopsy does not exist. Even when a postmortem examination is done, determination of chemical abnormalities may help establish the cause of death where the autospy findings are inconclusive or may validate the significance of recognizable organ changes. For this reason rather extensive studies have been made in the postmortem chemistry of blood. It has been found that abnormalities in concentrations of protein, calcium, urea nitrogen, creatinine, and bilirubin can be evaluated by study of the serum prior to the onset of intravascular hemolysis. Unfortunately there are many constituents in which postmortem changes occur rapidly and erratically so that extrapolation to probable antemortem values becomes impossible. Serum sodium and chloride concentrations begin to fall soon after death, but the rate of fall varies so markedly from individual to individual that it is impossible to detect electrolyte imbalance except in extreme cases and when the blood has been obtained very soon postmortem. Serum glucose will ordinarily decrease in the left heart and extremital vessels due to continued utilization by the red cells after death, but may be markedly elevated in cases where death results from asphyxia or is accompanied by a large output of epinephrine terminally. Glucose concentrations in blood obtained from the right heart or inferior vena cava frequently are found markedly elevated due to glycogenolysis in the liver but may be low in cases of starvation.

Because of the marked changes which occur in blood concentrations, other body fluids have been examined to determine whether a more accurate estimate of antemortem abnormalities would be possible. Cerebrospinal fluid has been rather extensively studied, but is frequently difficult to obtain and is commonly contaminated by blood in the process. Further, changes in the cerebrospinal fluid occur fairly rapidly and are quite erratic. For this reason there has been an increasing interest in the use of vitreous humor for chemical analysis.

The eyeball is isolated and well protected anatomically. As a consequence vitreous humor is usually preserved despite serious trauma to the head and is much less subject to contamination or putrefactive change than either blood or cerebrospinal fluid. Further it is easy to obtain by inserting a No. 20 gage needle into the center of the eyeball through the outer canthus of the eye and gently aspirating the vitreous humor with a small syringe. With care, approximately 2 ml of crystal clear fluid can be obtained from each eye. This provides sufficient material for determination of sodium, potassium, chloride, urea

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nitrogen, glucose, and alcohol. Such clear specimens frequently may be available even in bodies showing early decomposition in contrast to blood specimens where extensive hemolysis of the red cells is common. Vitreous humor is easy to handle in the laboratory paralleling in convenience the aqueous solutions commonly used for standards and controls. Most important it has been found that in contrast to blood and cerebrospinal fluid, chemical changes for many substances occur much more slowly in the vitreous humor enabling the investigator to determine the presence of antemortem abnormalities that cannot be detected by examination of any other body fluid to the best of our present knowledge.

It is the purpose of this paper to explain the normal values for ordinary constituents of vitreous humor which have so far been established, to show by case reports how determination of these substances has aided in establishing cause of death, and finally to show how continued studies of vitreous humor may prove of value in toxicological studies or aid in other facets of forensic medicine.

Normal Values of Common Constituents

Naumann [*1*] and Leahy and Farber [*2*] both made studies on the vitreous humor, determining certain normal values but did not concern themselves with changes caused by varying postmortem intervals. I utilized a technique whereby the vitreous humor was drawn from each eye at different times after death enabling me to determine whether changes in various constituents represented postmortem deviations or could be assumed to represent antemortem abnormalities. From these studies the following table of normal postmortem values was derived [*3*].

TABLE 1—*Postmortem chemistries of vitreous humor in normal humans.*

Mean postmoretem Interval, h Range	1¾ ½–2½	5¾ 3–10	17¼ 10½–29
Number of specimens	60	50	35
Sodium (mEq/l.) ± S.E. (mean) Range 1 S.D.	143 ± 0.52 135–151 4.0	143 ± 0.67 131–151 4.7	141 ± 0.76 131–150 4.4
Potassium (mEq/l) ± S.E. (mean) Range 1 S.D.	5.6 ± 0.09 4.2–7.2 0.7	6.8 ± 0.15 5.1–8.9 1.0	8.7 ± 0.19 6.9–11.5 1.1
Chloride (mEq/l) ± S.E. (mean) Range 1 S.D.	121 ± 0.76 108–132 5.9	119 ± 0.86 105–132 6.1	118 ± 1.16 104–130 6.8
CO_2 Combining Power (mEq/l) ± S.E. (mean) Range 1 S.D.	15 ± 0.50 6–27 3.9	15 ± 0.49 4–20 3.4	13 ± 0.66 5–21 3.9
Calcium (mg/100 ml) ± S.E. (mean) Range 1 S.D.	6.7 ± 0.05 6.0–8.0 0.4	6.8 ± 0.05 6.1–8.0 0.4	7.0 ± 0.09 6.1–8.4 0.5
Urea nitrogen (mg/100 ml) ± S.E. (mean) Range 1 S.D.	17 ± 0.99 6–40 7.6	17 ± 0.89 4–33 6.3	18 ± 1.23 3–30 7.5
Glucose (mg/100 ml) ± S.E. (mean) Range 1 S.D.	84 ± 5.21 37–180 40	66 ± 5.13 27–180 36	51 ± 4.77 18–106 28

The data from this table indicate that sodium, chloride, calcium, and urea nitrogen all remain stable in the vitreous for prolonged postmortem intervals so that marked variations from the figures given can be assumed to represent antemortem change. In contrast potassium and glucose show definite changes with increase in the postmortem interval making their interpretation more difficult, but still of value.

Electrolytes

By studying hospital patients in whom terminal antemortem concentrations were available it was established [*3*] that markedly elevated or depressed values of sodium and chloride reflected antemortem abnormalities in these constituents. Utilizing sodium as a primary indicator Coe found that all individuals having vitreous sodium concentrations over 155 mEq/l or under 130 mEq/l were obtained from individuals having a clinically manifest electrolyte imbalance. This has proven of value in several subsequent medical examiner's cases of which the following case reports are illustrative examples.

Case 1

One and two-year-old Negro children were found by friends alone in a home after the parents had not been seen for some time. The two-year-old youngster was still alive while the infant was dead in the crib. An autopsy on the infant revealed no anatomic abnormalities other than dehydration. Postmortem chemical determinations on the vitreous of the infant revealed a sodium of 170 mEq/l and a urea nitrogen of 58 mg/100 ml substantiating the pathologists suspicions of electrolyte imbalance and dehydration that had been formed from study of the living brother. When the parents were later brought to trial for neglect, the postmortem chemistries provided the most significant pathological evidence presented in court.

Case 2

A 33-year-old white housewife called her physician one morning complaining of nausea, vomiting, and itching. While talking to the doctor she suddenly began coughing and then failed to respond. The physician sent an ambulance to the house where the deceased was found dead. Autopsy revealed no satisfactory gross or microscopic anatomic cause of death. A duodenal ulcer was found with some evidence of pyloric obstruction. Toxicological studies were negative, but vitreous humor chemical determinations revealed a sodium of 120 mEq/l and a chloride of 87 mEq/l indicating a striking hyponatremia and hypochloremia.

The husband, who was away at the time of his wife's demise, was later questioned and stated that the deceased had vomited approximately 50 times in the twenty-four hours prior to demise and had lost some 10–15 pounds in the two weeks before death due to an inability to keep food down. While there may be question that the ulcer was totally responsible for the vomiting, the postmortem chemistries did establish a definite cause of death not demonstrable in the routine autopsy procedures.

Urea Nitrogen

Extensive studies [*3*] on both normal and uremic individuals demonstrated that the vitreous urea nitrogen closely parallels the blood urea nitrogen over all ranges of urea retention. Further it is by far the most stable of all postmortem constituents studied, showing in over 90 percent of the cases a variation of less than 3 mg/100 ml between

specimens drawn over 100 hours apart. Determination of vitreous urea nitrogen has enabled us to correctly state the cause of death in several cases not autopsied.

Case 1

A 75-year-old white male had been ill for several days. Police had been to see him but he refused medical attention and was found dead in his bed by a fellow roomer. There was no evidence or history of trauma and routine toxicological studies were negative. It was believed that the deceased had probably died of a heart attack until the vitreous humor chemical determinations were conducted. These revealed hypernatremia with a sodium of 168 mEq/l and marked uremia with a urea nitrogen of 207 mg/100 ml.

Case 2

A 68-year-old white male had been ill for several days causing both the clerk at the apartment where he lived and the police to encourage him to go to the hospital for medical attention. However, he refused. The caretaker found the deceased dead on the floor. There was no evidence of traumatic injury externally or upon X-ray examination and toxicological studies were negative. The vitreous humor revealed a urea nitrogen concentration of 204 mg/100 ml.

Case 3

A 76-year-old recluse was found dead in his home bound hand and foot to his bed by electrical cords. The house obviously had been ransacked and robbed. There were burns on the wrists and ankles, indicating the deceased had tried to extricate himself, but there was no other evidence externally or upon postmortem examination of any traumatic injury. Toxicological examinations were negative and no anatomic lesion to account for death could be found. Postmortem determinations on vitreous humor revealed evidence of severe hypernatremia, hyperchloremia, and marked urea nitrogen retention substantiating the impression that death resulted from dehydration when the deceased was unable to break loose from his bonds after the robbers had left his house.

Glucose

In contrast to earlier publications [*4*] Coe [*3*] established that glucose concentrations in the vitreous humor of normal individuals tended to fall with increasing portmortem interval. The rate of fall was frequently very erratic with precipitous drops to very low glucose concentrations occurring in a matter of a few hours in some individuals. As a consequence of this it is difficult to make a diagnosis of antemortem hypoglycemia, except under very limited circumstances. However, the demonstration of a normal glucose in the vitreous humor will exclude the possibility of hypoglycemia and this can be of great value in cases of known diabetics in which the terminal event is suspected of being a possible hypoglycemic reaction rather than some other cause.

In contrast to the difficulty in diagnosing hypoglycemia, examination of the vitreous humor is of great value in establishing the diagnosis of diabetic acidoses. We have a number of cases in the Hennepin County Medical Examiner's files in which this has been done. The following two case reports are illustrative.

Case 1

A 52-year-old white female recluse was found dead by the caretaker in the apartment where she lived. There was no known medical history. Autopsy and toxicological ex-

aminations were unrewarding, but chemical examination of the vitreous humor revealed a glucose concentration of 1100 mg/100 ml with a 4+ acetone reaction, establishing death as due to diabetic acidosis.

Case 2

A 59-year-old white female known diabetic who took 50 units of insulin every day, was found dead on the floor by the hotel manager after not having been seen for several days. External examination and X-ray studies revealed no evidence of traumatic injury. Toxicological examinations were negative. The vitreous glucose concentration was 893 mg/100 ml with a 4+ reaction for acetone indicating that the deceased was in severe diabetic acidosis.

Potassium

While potassium is found to vary after death making it impossible to utilize vitreous humor determinations to detect antemortem potassium abnormalities, mention should be made of the rather extensive studies into the use of vitreous potassium as a means of estimating the postmortem interval. It was first noted by Jaffe [*5*] and later substantiated by Adelson et al [*6*], Hansson et al [*7*], Hughes [*8*], Lie [*9*], and Sturner [*10,11*] that the potassium concentrations rise in a systematic fashion in the vitreous humor after death. Sturner and Lie both found such a close correlation between the vitreous potassium concentration and the postmortem interval that they believed the method could be used with a confidence limit of ±5 h. Environmental factors did not seem to influence the results and Sturner stated that the standard error did not increase as the death interval increased. Adelson et al, and Hughes, Hansson et al, and Coe all substantiated that the mean rise in any large group was arithmetic with time and essentially independent of external factors. However, there was such individual variation that the confidence limit of the method exceeded ±10 h in the first day after death. Hansson et al and Coe further showed that the standard error then continued to increase with longer postmortem intervals. Nevertheless the method can be of some help in estimating the time of death in cases where the classic indications of temperature, lividity, rigor, and decomposition give conflicting information.

Toxicology

Recently investigations have been initiated to determine the suitability of the vitreous humor as a substance for toxicological examination. Four studies have been made of vitreous humor for alcohol demonstrating this to be an excellent medium for its determination by any of the standard procedures now in use. While the ratio between vitreous and blood alcohol concentration has shown variation between the four groups so far reported [*12–15*] Coe has found that in his series of cases the ratio of blood to vitreous alcohol equals 0.89, that is, that the blood alcohol concentration equals 0.89 × the vitreous humor alcohol concentration.

The advantage of using vitreous humor for alcohol determination is the ease of obtaining the specimen in an uncontaminated state in contrast to the blood where traumatic injuries have frequently results in rupture of internal organs. Further because alcohol is usually interpreted in relation to whole blood, problems arise in the utilization of postmortem blood because of intravascular clotting which can cause the hematocrit of the specimens to vary greatly from what they would have been in the live individuals.

Research is now underway to determine the suitability of utilizing vitreous humor for other toxicological substances. It has been found by Felby and Olsen [*16*] that barbiturates will be present in the vitreous humor, although in a lower concentration than in the blood of the same individuals.

Conclusion

The vitreous humor is an easily obtainable body fluid less subject than blood or cerebrospinal fluid to contamination or putrefactive changes. It has been found suitable for a number of chemical determinations and limited toxicological studies. These can be utilized in helping to determine the cause of death, estimating the postmortem interval, or establishing body concentrations of certain drugs when blood specimens are unavailable or contaminated.

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