Effect of Caffeine on Post-Mortem Blood Coagulation

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The coagulation rate of blood can be intrinsic in determining the time of death of a subject. The purpose of this experiment is to determine the effect that caffeine has on the coagulation of post-mortem blood in order to assist in vital forensic data. The experimental design included use of 200 mg caffeine pills, Stay Awake by Equate, and mix the crushed pill with blood from *Bos taurus*. Blood temperature was considered and two experiments conducted with a dependent variables of room temperature and the temperature of the refrigerator utilized. Half of the samples contained caffeine, while the others did not. The rate of coagulation was measured with data being recorded every hour, then once at the 24-hour, and once at the 48-hour mark. Refrigerated blood, regardless of caffeine addition, had very little coagulation after 24 hours. Colder temperatures appear to slow coagulation of blood post mortem, even when there is no circulation of blood.

Keywords: coagulation, caffeine, temperature, *Bos taurus*

The rate of coagulation of blood can be intrinsic to determining the time of death of a subject. Caffeine is known to be a blood thinner, thereby potentially delaying the coagulation of blood. The conduction of this experiment was meant to determine whether caffeine impacts on coagulation, when dissolved in blood. Upon conducting this study, it was understood that caffeine "produces a rise in the free fatty acid levels of the blood," (Bellet 1967) which would lead to higher rates of clotting, thereby influencing the hypothesis higher levels of caffeine will delay the clotting of blood. However, studies have shown that higher levels of any form of drug do not necessarily appear in the same high concentrations post mortem. It is speculated that there is a process of "postmortem redistribution…and drug metabolism immediately after death" (Alicot 2003) that could potentially fully dispose of the caffeine before postmortem processes such as coagulation occur. While this experiment combined the blood and caffeine postmortem, in an ideal scenario it can be speculated that the caffeine would have been disposed of by the body if ingested *in vivo.* The clotting of blood post mortem is believed to be a result of an increase in red blood cells, also known as hematocrit. Once blood stops flowing, there is "a rise in the hematocrit up to 80%," (Jackowski 2005) until it reaches a point at which the blood is too thick to flow- this is what forms the coagulate. Since the blood used in this experiment was collected postmortem, the rate of coagulation is expected to be higher since it has obviously been without circulation for an extended period of time. Instead of human blood being used in this study, blood collected from a cow was used instead. Cow blood is similar enough to human blood that this alteration should not bare any significant errors in the testing of this experiment. In fact, cow blood is so similar to human blood that it has been used in the medical field for "blood transfusions for accident victims" (Altman 1991). Therefore, the rate of coagulation or reaction with caffeine should bare resemblance to how human blood would react.

Materials and Methods

Blood was collected from Rosenthal Meat Market in a plastic water bottle container. Half of the blood contents were then transferred to a glass mason jar, and finally were transferred into two different paint containers. There were six paint cups on each container, and three of these cups were filled with 200mg of caffeine powder. The caffeine powder was collected from a crushed *Equate Stay Awake* caffeine pill. Each cup was given approximately one tablespoon of blood. The blood was then inverted several times to full mix with the caffeine powder. One container was placed in a refrigerator, the second was kept out on a shelf. Initially the blood was checked every hour, and then time between each observation was extended to every few hours. After the 24-hour mark, the blood was only observed once more at 48 hours. There were several errors to this method of experimentation. The first being that the day the blood was collected, and the day the experiment was done were three days apart. This led to substantial clotting of the blood inside of the alter bottle, and while the clot was dissolved as much as possible, the blood was still separated by the point of experimentation. Second, one tablespoon of blood is must smaller than the optimal amount of blood to be used in this type of experiment. And finally, there was no real way to measure the coagulation of blood once it was in the cups. All observations were made by the naked eye alone.

Results

When the blood sample was refrigerated and caffeinated, in 24 hours it had slight changes. In a 48-hour period, it was slightly coagulated. When refrigerated and not caffeinated, similar results presented. When the blood sample was maintained at room temperature and caffeinated, a caffeine layer formed after 24 hours After 48 hours, the coagulate mixed with the caffeine. Lastly, when an un-caffeinated blood sample was held at room temperature, there was noticeable layer separation after 24 hours between the blood and the caffeine. In 48 hours, the layers were identifiably completely separated.

Figure 1: Data Summary of Blood Clots

Blood Treatment	24 Hours	48 Hours
Refrigerated and Caffeinated	Little to no change, blood has not separated	Slight coagulation over layer of caffeine formed at bottom, little separation
Refrigerated and Regular	Little to no change, blood has not separated	Little coagulation but blood appears lighter, small coagulum pooled at bottom
Room temperature and Caffeinated	Blood has begun to separate, caffeine formed layer at bottom	Thick coagulate mixed in with caffeine, substantial liquid layer at top
Room temperature and Regular	Noticeable separation, coagulate pooled at bottom	Coagulate floating in middle of blood with liquid layer on top, noticeable separation

Discussion:

Refrigerated blood, regardless of caffeine addition, had very little coagulation after 24 hours. Colder temperatures appear to slow coagulation of blood post mortem, even when there is no circulation of blood. Ideally, "whole blood can be stored at 4-8 °C for up to 24 hours before the serum is separated" (World Health Organization 2012), which was reflected in the data collected. The refrigerator used in this experiment did not have an accurate thermometer, however it was approximately 1.6 ° C. For the room temperature samples, both the caffeinated and un-caffeinated experienced noticeable separation, and coagulum's settling at the bottom of the cup. The caffeine pooled at the bottom of the sample, with a coagulum coating it. The caffeine appears to not have fully dissolved, and whether that is due to too small a blood sample, or lack of circulation is undetermined. In a similar study, with whole blood kept at room temperature, "plasma coagulation factor activity was surprisingly modest compared to literature values" (Hughes 2007). It seems that the rate of coagulation for this experiment is on par with others done at different scales, even though there are several differential factors. After 48 hours, the refrigerated samples began showing slight separation, roughly the same level of separation in both samples. The coagulate again pooled at the bottom of the cups, one coating the undissolved caffeine. The room temperature samples at 48 hours again had substantial changes. The caffeinated sample had a thick coagulate mixed in with the undissolved caffeine, and had a liquid portion of blood filling the rest of the cup. The liquid portion of blood appeared extremely watery, and was a very light-colored red. "Serum is the liquid that remains after the… blood has clotted and retracted into a clump" (Lyle 2004), and is what appeared in both room temperature samples. The un-caffeinated blood had the coagulate floating in the middle of the sample, with light colored liquid surrounding it. Typically, the effect of blood coagulation would be performed *in vivo*, as the blood would ideally be continuously circulating. However, in similar studies conducted where the blood was still in a living organism, caffeine had "no effect of… clotting factor VII activity" (Bak 1990) or any other hemostasis function. In fact, caffeine is a recommended tool in the prevention of several health problems that result from the buildup of fatty acids. Caffeine can "keep blood clots from forming" (Adams 2013), which supports the hypothesis made at the beginning of the experiment. Since caffeine acts as a blood thinner, it is more difficult for clots to form and create a thick jelly like substance. If the study were to be done again, it would be taken into consideration the difficulty of finding concentrations of drugs in the blood postmortem, then compared to other bodily fluids. Bile and urine have been proved to be much more effective in depicting drug concentrations ante mortem, "as bile (is a) major excretion route of a spectrum of drugs and their metabolites", and "Urine is a conventional material for drug screening and pharmaco-/toxicokinetic analysis" (Tominaga 2016). Since blood needs to be constantly circulating in order to process and disperse drugs, it is difficult to determine the metabolization and concentration levels of blood post mortem.

References:

Adams, S. 2013. Daily coffee 'lowers stroke risk'. The Telegraph. (http://www.telegraph.co.uk/news/health/news/9930710/Daily-coffee-lowers-stroke-risk.html).

Altman, L. K. 1991. U.S. Approves Test of Blood Substitute From Cattle. The New York Times. (http://www.nytimes.com/1991/02/25/us/us-approves-test-of-blood-substitute-from-cattle.html).

Bak, A., H. V. Vliet, and D. Grobbee. 1990. Coffee, caffeine and hemostasis: results from two randomized studies. Atherosclerosis. 83: 249–255.

Bellet, S. 1965. The Effect of Caffeine on Free Fatty Acids. Archives of Internal Medicine. 116: 750.

Hughes, J., V. Macdonald, and J. Hess. 2007. Warm storage of whole blood for 72 hours. Transfusion. 47: 2050–2056.

Jackowski, C., M. Thali, E. Aghayev, K. Yen, M. Sonnenschein, K. Zwygart, R. Dirnhofer, and P. Vock. 2005. Postmortem imaging of blood and its characteristics using MSCT and MRI. International Journal of Legal Medicine. 120: 233–240.

Lyle, D. P. 2016. Forensics for dummies. For Dummies, a Wiley Brand, Hoboken, NJ.

Pélissier-Alicot, A.-L., J.-M. Gaulier, P. Champsaur, and P. Marquet. 2003. Mechanisms Underlying Postmortem Redistribution of Drugs: A Review. Journal of Analytical Toxicology. 27: 533–544.

Region, E. 2004. Surveillance guidelines for measles and congenital rubella infection in the WHO European Region. Weekly releases (1997–2007). 8.

Tominaga, M., T. Michiue, S. Oritani, T. Ishikawa, and H. Maeda. 2016. Evaluation of Postmortem Drug Concentrations in Bile Compared with Blood and Urine in Forensic Autopsy Cases. Journal of Analytical Toxicology. 40: 367–373.