Original Article

Effects of Storage Conditions on Predetermined levels of Carboxyhaemoglobin in the Postmortem Animal Muscle Tissue

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20 samples of post mortem sheep muscle were exposed to smoke for variable duration and were stored under varying conditions. Carboxyhaemoglobin levels were estimated in pre storage samples and after 24, 48 and 72 hours in the stored samples. There was a small fall in carboxyhaemoglobin levels when samples were stored at 4°C in the dark. There was a marked fall in carboxyhaemoglobin levels when samples were exposed to atmosphere in transparent bottles. Higher the initial levels of carboxyhaemoglobin in the tissues, higher was the decay rate after exposure at atmosphere.

Keywords :- Muscle Carboxyhaemoglobin, Aircraft accident investigation.

Introduction

Estimation of carboxyhaemoglobin levels in the blood or postmortem tissues of aircraft accident victims forms an important aspect of aircraft accident investigation. Many aircraft accidents particularly those involving combat aircrafts, lead to total disintegration of bodies with the result that blood may not always be available for toxicological analysis and the analyst may have to depend on only a piece of the disintegrated tissue for carboxyhaemoglobin estimation. Moreover, carboxyhaemoglobin being photolabile, its levels are influenced by various environmental factors like sunlight and variable temperature.

The present study was, therefore, undertaken to determine the effects of storage conditions on predetermined levels of caboxyhaemoglobin in the postmortem sheep muscle tissue.

Materials and methods

Twenty samples of Postmortem sheep muscle tissue exposed to smoke for variable duration were utilized for studying the effects of various storage conditions. The smoke was produced in a fabricated smoke-chamber by burning rags and fragments of plastic and foam material alongwith the aviation fuel to simulate conditions as akin to aviation environment as possible.

Ten samples were exposed to smoke/fire for half an hour (sample Nos 1-10) and another ten for one hour (Sample Nos11-20). After the exposure, blood was eluted from each sample in 0.1% Liquor ammonia and carboxyhaemoglobin estimation carried out using digital reading Beckman series 34/UV/visible spectrophotometer.

Each sample was then divided into two portions, one of which was exposed to atmosphere in transparent bottle and other was stored at 4°C in dark coloured bottle for 72 hours and carboxyhaemoglobin level estimated at intervals of 24, 48 and 72 hours.

Results

Table I Carboxyhaemoglobin levels in postmortem animal muscle tissue samples before and after storage at 4°C

Sample No.	COHb% before storage	COHb% after storage at 4°C		
		24 hours	48 hours	72 hours
1	4.26	4.20	4.20	4.00
2	4.28	4.20	4.26	4.10
3	1.40	1.50	1.36	1.06
4	5.40	5.28	5.16	5.00
5	6.50	6.42	6.00	5.86
6	5.44	5.40	5.40	5.40
7	3.46	3.50	3.20	3.00
8	4.20	4.00	3.56	3.16
9	5.48	5.40	5.00	4.98
10	5.00	4.20	4.10	3.96
Mean	4.54	4.41	4.22	4.05
SD	1.35	1.28	1.32	1.40
Range	1.4 - 6.5	1.5 - 6.42	1.36 - 6.0	1.06- 5.86

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The mean carboxyhaemoglobin level of 4.54% decreased to 4.05% with a SD of 1.40 after storage at 4°C for 72 hours.

Table II Carboxyhaemoglobin levels in postmortem animal muscle tissue samples before and after exposure to atmosphere

Sample No.	COHb% before exposure	COHb% after exposure to atmosphere		
		24 hours	48 hours	72 hours
1	4.23	0.00	0.00	0.00
2	4.28	2.02	1.50	0.50
3	1.40	1.00	0.00	0.00
4	5.40	3.22	2.62	1.06
5	6.50	4.22	3.05	1.22
6	5.44	3.16	2.82	0.00
7	3.46	3.00	2.02	1.32
8	4.20	0.00	0.00	0.00
9	5.48	4.12	2.54	2.00
10	5.00	3.80	3.76	1.24
Mean	4.54	2.45	1.83	0.73
SD	1.33	1.53	1.32	0.69
Range	1.4 - 6.5	0.0 - 4.22	0.00 - 3.76	0.00- 2.00

The mean carboxyhaemoglobin level of 4.54% decreased to 0.73% with a SD of 0.69 after exposure to atmosphere for 72 hours.

Table III Carboxyhaemoglobin levels in postmortem animal muscle tissue samples before and after storage at 4°C

Sample No.	COHb% before storage	COHb% after storage at 4°C		
		24 hours	48 hours	72 hours
11	6.38	6.40	6.30	6.36
12	5.84	5.82	5.84	5.84
13	7.86	7.00	7.05	7.00
14	6.80	6.12	5.78	5.00
15	5.96	5.96	5.90	4.78
16	5.90	5.06	4.92	4.80
17	6.10	6.00	6.12	5.18
18	5.68	5.70	5.86	5.72
19	6.28	6.00	5.10	4.94
20	5.40	5.68	5.50	5.38
Mean	6.22	5.97	5.83	5.50
SD	0.66	0.48	0.59	0.70
Range	5.40- 7.86	5.06- 7.00	4.82 - 7.05	4.78- 7.00

The mean carboxyhaemoglobin level of 6.22% decreased to 5.50% with a SD of 0.70 after storage at 4°C for 72 hours.

Table IV Carboxyhaemoglobin levels in postmortem animal muscle tissue samples before and after exposure to atmosphere

Sample No.	COHb% before exposure	COHb% after exposure to atmosphere		
		24 hours	48 hours	72 hours
11	6.38	4.50	2.10	1.02
12	5.84	4.66	3.20	1.00
13	7.86	5.28	2.34	2.00
14	6.80	2.48	0.00	0.00
15	5.96	2.64	1.12	0.00
16	5.90	2.88	0.00	0.00
17	6.10	3.84	2.18	2.04
18	5.68	2.46	1.00	0.00
19	6.28	6.28	3.42	2.10
20	5.20	3.76	1.72	0.98
Mean	6.22	3.88	1.17	0.91
SD	0.66	1.23	1.12	0.85
Range	5.40- 7.86	2.48- 6.28	0.00 - 3.42	0.00- 2.10

The mean carboxyhaemoglobin level of 6.22% decreased to 0.91% with a SD of 0.85 after exposure to atmosphere for 72 hours.

Discussion

The importance of carboxyhaemoglobin estimation particularly that of its grossly elevated levels in the blood or tissues of aircraft accident victims, can not be overemphasised and leaves no doubt about the antemortem exposure of victims to carbon monoxide[1-4]. The interpretation of marginal levels of carboxyhaemoglobin which may give an entirely new direction to investigations in a fatal aircraft accident becomes difficult at times, not only because carboxyhaemoglobin levels of as much as 8-10% may be found in the blood of chronic heavy smokers[3,5-7] but also because carboxyhaemoglobin levels may deteriorate subsequent to death due to factors like time-lag and exposure to atmosphere and sunlight[8-11].

While studying the effects of storage at 4°C on predetermined levels of carboxyhaemoglobin, we have found that, although, the mean carboxyhaemoglobin level in tissue samples exposed to smoke for half an hour has fallen from 4.54% to only 4.05% after storage for 72 hours, but even this decrease is statistically significant. Similarly, the decrease in carboxyhaemo- globin levels from 6.22% to 5.5% in one hour

smoke-exposed samples after storage at 4°C for 72 hours, has also been found to be statistically significant.

Both sets of samples have shown a very highly significant deterioration in carboxy-haemoglobin levels when exposed to atmosphere in transparent bottles with virtual disappearence of carboxyhaemoglobin within 72 hours. Another significant finding from our data is that, higher the initial level of carboxyhaemoglobin in the tissues, higher is the decay rate after exposure to atmosphere, a finding which is in agreement with that of Camps[9] and Hyde et al[11].

It, therefore, becomes amply clear that an utmost restraint must be exercised while interpreting the marginally high levels of carboxyhaemoglobin in blood/tissue samples of aircraft accident victims and no efforts must be spared to relate these marginally high levels to circumstantial evidence of the accident and the storage conditions to which samples have been exposed prior to analysis.

Moreover, considering the diversity of factors influencing carboxyhaemoglobin levels, it can be emphasised that the muscle tissue samples for Carboxyhaemoglobin estimation must be collected with the least possible delay after the accident and then immediately stored at 4°C in dark coloured bottles, so as to ensure minimum decay before analysis.

Conclusion

Carboxyhaemoglobin levels in the tissues are influenced by a variety of environmental factors and storage conditions, to which due consideration must be given before interpreting their significance in the investigation of aircraft accidents. The study has the limitation of using animal muscle tissue which may differ with human muscle tissue because of species differences in the affinity of various haemoglobins to carbon monoxide.

REFERENCES

1. Blackmore DJ : Aircraft accident toxicology, U.K. Experience 1967-1972. Aerospace Med. 1974; 45 (8) : 987-994

2. Dille JR, Linder MK : The effects of tobacco on aviation safety. Aviat. Space Environ. Med. 1981; 52 (2) : 112-114

3. Dominguez AM, Glantz N, Goldbaum LR : Carbon monoxide determination in aircraft accident fatalities. Aerospace Med. 1959; 30 : 711-715

4. Nuttal JBJ : Toxic Hazards in the Aviation Environment. Aviat. Med. 1958; 29 : 641-649.

5. Mcfarland RA : Milit. Med. 1955; 166 : 423.

6. Stembridge VA : Jt. Comm. Aviat. Path, Memo No.4, 1959

7. Stembridge VA, Craft WM, Townsend FM : Medical Investigation of Aircrafts Accidents with Multiple Casualties. J. Aviat. Med. 1958; 29 : 668-675

8. Blackmore DJ : The determination of carbon monoxide in blood and tissues. Analyst 1970; 95 : 439-456

9. Camps EF : Transportation accidents In Gradwohl's legal medicine. John Wright and Sons Ltd. Briston, 1968; 2 : 397

10. Gettler AO, Freimuth HC : The carbon monoxide content of the blood under various conditions. Am. J. Clin. Path. 1940; 10 : 603

11. Hyde TW, Mellov LB, Raphaer SS : Elementary Toxicology and ultromicro analysis in Lynch's Medical Laboratory Technology 1976 : 396-397.