Original Article

Laboratory Studies on Avian Blood under Simulated Crash Conditions

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Birds pose a constant threat to aircraft and are one of the common flight safety hazards. Incidents/accidents attributable to bird strike are still very high all over the world. In such eventualities, identification of avian blood/tissue from aircraft is of paramount importance. However, due to availability of very scanty sample, which is heavily contaminated and poorly preserved, usual simple laboratory procedures are full of discrepancies. The utility and efficacies of various laboratory procedures like chemical examination of blood by benzidine/orthotoluidine test, microscopic examination of blood for nucleated red blood cells, haemochromogen crystals test (Takayama method), blood urea nitrogen/uric acid ratio and amino acid pattern by thin layer chromatography under simulated aircrash situations have been evaluated. The utility of Benzidine Test is very much limited but the presence of blood can be confirmed by the formation of haemochromogen crystals. Avian red cells are discernible up to 48 hrs of exposure to simulated aircrash situations. BUN/Uric Acid ratio and amino acid pattern of avian blood is marginally different from the human blood and require further elaborate study.

Keywords : *Bird Hit Investigation, Aircraft Accident Investigation, Haemochromogen crystals, Amino acid pattern.*

Birds pose a constant threat to aircraft and are one of the common flight safety hazards[1]. The gravity of the situation can be realised by the fact that US Air Force alone averages about 2000 bird strikes a year[2]. There were 2559 bird strikes, costing $ 242,628,244 and 5 fatalities that were reported to Bird Aircraft Strike Hazard (BASH) team in USA during 1987[3].

In the IAF too, bird strikes have resulted in a loss of Rs.10.63 crores (1981-82), 9.09 crores (1982-83) and 23.82 crores (1983-84) as well as irreparable loss of human lives[4]. A study carried out by Aeronautical Development Agency on bird strike incidents of fighter aircraft for the period 1975-86 revealed that of 431 bird strike incidents, damage to aircraft occurred in 409 cases (94.8 percent). Loss of aircraft was reported in 34 cases (8.3 percent) out of total of 409 bird strikes. This study did not include accidents due to bird strike[5].

In cases of aircraft accidents due to bird strike, identification of avian blood/tissue from aircraft is of paramount importance. It is usually based on circumstantial evidence provided by the pilot, supported by damage to aircraft and the presence of feathers or blood or any avian tissue which can be recognised. The former is applicable in non fatal aircraft accidents, where the aircraft is not abandoned after strike.

In fatal aircraft accident investigation or when aircraft is abandoned after accident, establishment of bird strike is very difficult. Besides, in many cases of reported engine failures where exact cause cannot be found, bird ingestion into the engine resulting in failure needs exclusion .This may help in the investigation as it would delink this cause of failure from other suspected mechanical failures and economise on the investigation effort.

However, identifying and establishing of bird's tissue/blood gathered from the remains of a wrecked aircraft body is problematical. This material is usually collected after a lapse of considerable time, is grossly contaminated and subjected to vagaries of weather. In many cases, it is exposed to intense heat produced as a result of post crash fire.

This study was, therefore, aimed at studying procedures like chemical examination of blood by benzidine test, microscopic examination of blood for nucleated red blood cells, haemochromogen crystal test (Takayama method), blood urea nitrogen/uric acid ratio and amino acid pattern by thin layer chromatography,

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in fresh avian and human blood. These procedures were evaluated to assess their efficacies under simulated aircrash situations as well.

Material and Methods

For study of avian blood, young fowls (Gallus gallus) of white leghorn class were procured and housed in the animal house of Institute of Aerospace Medicine (IAM), IAF. After raising them under standardised dietetic regime for 16 weeks, procedure for collection of blood by venepuncture on the undersurface of wings was standardised. 5-10 ml of blood was collected and subjected to a group of tests immediately after collection and later after drying, in order to simulate samples from an aircrash situation.

Fresh blood samples were examined microscopically and biochemically to standardise the normal avian blood presentation. To study dried blood, samples obtained after bleeding were immediately smeared on a part of airframe and glass plates. These were kept in open to expose to ambient temperature, sunlight, humidity and contaminated to simulate aircraft accident situation and tested after a period of 2 hrs, 48 hrs, 72 hrs and 7 days. Glass plates, duly smeared with blood were kept in hot air oven at 90°C for 4 hours and various tests were carried out to assess their efficacy/reliability when sample is exposed to intense heat as may often be encountered in immediate post crash phase.

Following tests were carried out on these samples of blood :-

(a) Chemical examination by benzidine test[6,7]

(b) Microscopic examination for nucleated red blood cells

(c) Haemochromogen crystal test (Takayama method)[6,7]

(d) Blood urea nitrogen/uric acid ratio[8,9]

(e) Amino acid pattern by thin layer chromatography[10,11]

Human blood from healthy volunteers reporting to Medical Evaluation Centre, IAM was also studied simultaneously under identical circumstances and comparison made at various stages.

Blood samples from commonly available birds viz Ducks (Cairina Mosohata) and pigeons (Streptopelia turtur) were also subjected to the above mentioned group of tests.

Results

Fresh avian and human blood was examined to familiarise ourselves with normal morphology of red blood cells, relevant biochemical profile and amino acid pattern. Results are given in Table I.

Table I : Results of Various Procedures Carried out on Fresh Blood

Test	Avian blood	Human blood
Benzidine Test	Intense blue colour	Intense blue colour
Microscopic Examination	Elliptical pink cells having ovoid nucelus	Round, pink discs, Non-nucleated.
Haemochromogen Crystals	Orange red crystals	Orange red crystals
BUN/Uric Acid ratio	1.8:1 upto 2.6:1	4.5:1 upto 5.6:1
Amino Acid pattern	Six bands	Six bands

Red blood cells of birds do not show a distinct nucleus when examined (without staining) under the microscope. These cells appear ellliptical/ovoid and do not form rouleaux. They are light pink in colour. Human red cells appear as non nucleated round discs, forming rouleaux at places. However, Avian Red blood cells when examined after staining with methylene blue show centrally placed, dark blue, ovoid nucleus with well defined nuclear membrane. The nucleus covers almost the whole RBCs leaving a thin rim of cytoplasm around it as shown in Fig 1.

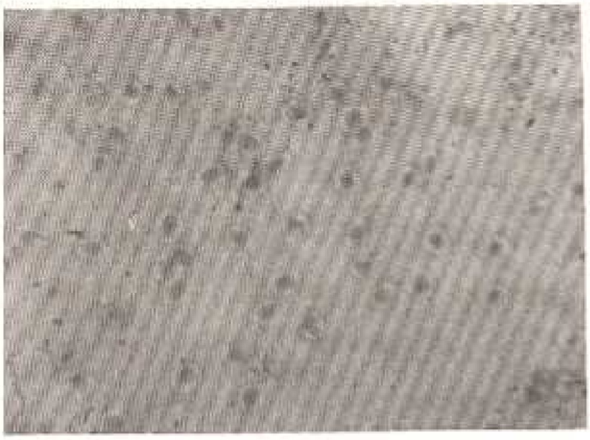
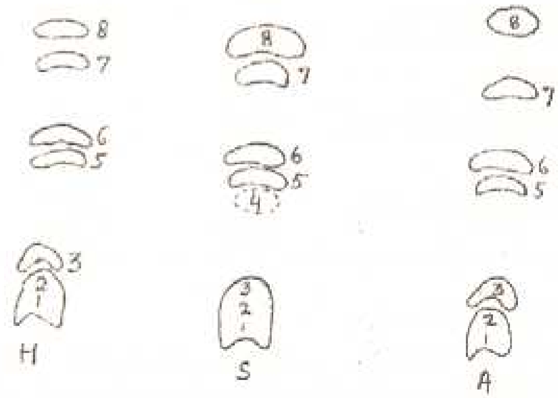


Fig 1. Photomicrograph showing nucleated red blood cells after staining (X 450)

Haemochromogen crystals develop immediately and appear as orange red elongated blade shaped structures of variable sizes, joined together at places thus giving feathery appearance. The morphology and colour reaction of crystals is the same in human and avian blood. Haemochromogen crystals formed from avian blood are shown in Fig 2.

Fig 2. Photomicrograph showing haemochromogen crystals formed from avian blood (X 100)

Blood urea nitrogen/uric acid ratio estimated in fresh sample shows a distinct difference between the avian and human blood. Amino acid pattern on Thin layer chromatography (TLC) shows six bands of amino acids in avian and human blood corresponding to histidine and arginine together, followed by glycine, tyrosine, methionine, phenyl alanine and leucine with histidine closest to the point of application of sample. There are no distinct differentiating features between the two patterns except that leucine movement appears marginally more in case of avian blood as compared to human blood. Schematic representation of various amino acids separated is shown in Fig 3.



H- Human; S-Standard; A-Avian; I-Histidine; 2-Arginine; 3-Glycine; 4-Proline; 5-Tyrosine; 6-Methionine; 7-Phenyl Alanine; 8-Leucine

Fig 3. Amino Acid Pattern

Effects of exposure of avian blood to various simulated crash conditions are shown in Tables II and III.

Table II : Effects of Exposure of Blood to Intense Heat (90°C for 4 hrs) on a Glass Slide

Test	Avian blood	Human blood
Benzidine Test	+++	+++
Microscopic Examination	Cell debris and few bare nuclei seen.	Cell debris seen
Haemochromogen Crystals	Seen	Seen
BUN/Uric Acid ratio	1.0:1 - 1.2:1	4.6:1 - 4.8:1
Amino Acid pattern	Six bands	Six bands

Table III : Effects of Exposure of Avian Blood to Sunlight Temperature, Air and Humidity For 2 hrs, 24 hrs 48 hrs, 72 hrs and 7 Days

Test	2 hrs	24 hrs	48 hrs	72 hrs	7 days
Benzidine Test	+++	+++	+++	+++	+++
Microscopic Exam	Cells Seen	Cells Seen at Periphery	Cells Seen at periphery	Cell debris seen	Cell debris seen
	Nucleus Seen	Nucleus Seen	Nucleus Seen	Nucleus Not Seen	Nucleus Not Seen
Haemochromogen Crystals	Seen	Seen	Seen	Seen	Seen
BUN/Uric Acid Ratio	1.8:1 -2.6:1	1.3:1 -2.0:1	1.3:1 -2.0:1	1.3:1 -1.8:1	1.3:1 -1.8:1
Amino Acid Pattern	Six bands	Six bands	Six bands	Six bands	Six bands

Discussion

In aircraft accident situations, many substances encountered during investigation may produce stains which may be indistinguishable from blood when examined by naked eye only. Aviation fuel/oils mixed with soil and impression produced by bark of a tree may produce brownish stains like that of blood[12]. Thus confirmation of blood by specific tests is a very important initial requirement.

It was observed that chemical examination of blood by benzidine test can be used as a screening test only. If found negative, the presence of blood is ruled out. When positive, presence of blood need to be confirmed by other specific tests as it gives false positive with a host of other materials[12]. Its utility in aircraft accident investigation is very much limited.

Our findings are in agreement with that of Richey et al[12] who could identify avian red cells after exposure to air turbulance for 2 hrs 30 mts on a T-38 aircraft which flew to a height of 42,000 ft and at speeds upto 1.2 Mach. They did not extend their study to prolonged exposures and intense heat as carried out in present study. They also observed that red cells are better discernible when examined after suspending the dry blood flakes in distilled water and not isotonic saline. It is possible that shrunken RBC in dried blood swell after imbibing distilled water and thus appear clearly.

Haemochromogen crystals could be produced with ease from very minute quantities of blood retrieved after exposure to various vagaries of weather and intense heat. The presence of blood can be confirmed by the formation of these crystals. The same has been the findings of many other workers[6,7,12,15]. The sole limitation of this test is its inability to differentiate between avian and human blood. Our findings are in partial agreement with Stork[16] who found BUN/Uric acid ratio of 1.8:1 - 3.49:1 in avian tissue and 5.52:1 - 27.18:1 in bat tissue. He did not study the changes in blood/tissue urea nitrogen and uric acid after exposure to various vagaries of weather.

The amino acid pattern obtained did not reveal any significant differences except that leucine movement appeared marginally but consistantly more in avian as compared to human blood. The amino acid pattern in different birds studied looked alike and there was no change of pattern in samples obtained after simulated aircrash situations. There were no gross quantitative differences in amino acids either. Our study is in partial agreement with Laham[17] who carried out amino acid analysis in seven cases of suspected bird strike using a "Chromobed resin" (Technician) system. He could not get an unequivocal amino acid pattern and emphasised the need for a quantitative estimation and more studies in the field. We also feel that more elaborate study is required in this field.

In the present study, various conventional methods for detection and confirmation of blood specially from dried samples were evaluated. The utility and reliability of various simple tests which can be carried out for differentiation of avian from human blood were explored. The efficacies and limitations of these methods in simulated aircrash situations were evaluated.

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