Toxicological and Pathological Findings

Dr. Michael Krämer
Flugmedizinisches Institut der Luftwaffe
Rechtsmedizin und Flugunfallmedizin
Postfach 1264 KFL
82242 Fürstenfeldbruck

FlMedInstLwAbtV@bundeswehr.org

SUMMARY

When investigating a fatal aircraft accident, the medical experts must also consider the requirements of the local public prosecution. The peculiarities in the toxicological and histological examination result on the one hand from the complexity of the matter and on the other hand from the elevated demands of conclusiveness and the variety of the examinations to be performed. The answering of the relevant questions regarding the aircraft accident investigation in connection with the critical assessment and interpretation of the findings makes highest demands on the investigators. The specific aspects and pitfalls regarding the assessment of the postmortal alcohol detection, the diagnostics of chronic alcohol abuse, the analysis of POL substances and combustion gases, the diagnostics of disturbances of the glucose metabolism and the microscopic examination of the heart will be discussed.

1 INTRODUCTION

According to STANAG 3318, aircraft accident investigation must enable answering the question whether the fitness for flying duty of the crew members was hampered by alcohol, medication, drugs, or other toxic substances such as POL (inhalation of hydraulic oil, kerosene etc.) incineration or exhaust gases and the thermal decomposition products of organic material. Moreover, the possibility of pathological changes having affected the airworthiness of crew members must be investigated and if such changes are due to acute or latent diseases.

Since the public prosecution of the various nations is the competent authority for the investigation of cases of unnatural death attending to the question of responsibility, any case requires the cooperation of the Federal Ministry of Defense with the respective German local prosecutor. Therefore, basic criteria for the liability of the methods of investigation must be observed.

Several peculiarities of the toxicological investigation procedures which are applied for aircraft accident investigation and several aspects of postmortal biochemical methods will be discussed in the following, possibly in conjunction with pathological findings.

2 CONSIDERATION OF THE MATERIAL TO BE INVESTIGATED AND THE NECESSARY SYMBIOSIS OF PATHOLOGIST AND TOXICOLOGIST:

The members of the aircraft accident crash group take samples for analysis at the site of the accident or in the mortuaries where the autopsy is carried out. Then the samples are transferred refrigerated or frozen to

Paper presented at the RTO HFM Lecture Series on "Pathological Aspects and Associated Biodynamics in Aircraft Accident Investigation", held in Madrid, Spain, 28-29 October 2004; Königsbrück, Germany, 2-3 November 2004, and published in RTO-EN-HFM-113.

the precincts of the Division Aircraft Accident Investigation to be analyzed. According to the principle "chain of custody", registration, receipt, treatment, and transfer of the material to the individual specialists, places where samples are kept, different phases of analysis are all recorded. This complete documentation enables to find out at any time who handled the material at what time and where. So, falsifications, confusions or willful manipulations can be excluded. If this documentation is missing, it cannot be re-enacted, if any substances and their concentrations which are detected at a later stage were at all relevant rendering impossible any final objective interpretation. If such conditions are not complied with, no disciplinary or criminal consequences can be drawn. The pathologists who know the conditions of the crash site and the particular conditions of the bodies - determine - together with the toxicologist - the choice of the samples and their treatment, the extractions, and the examination techniques. They have to consider the evidential value of the method applied and its specificity and accuracy. As a rule, blood, urine, hairs, tissue samples of the brain, heart, liver, kidneys, fatty tissues, etc. are tested for alcohol, medications and drugs. Not only the qualities of the specific substances, or the catabolic decomposition products are determined by two independently applied methods, but also their individual quantities are identified by two different analysts. As a rule, the quantitative result is the total of the results of several individual tests. Then an average value is calculated. According to the pertinent regulations, the individual results cannot but very slightly deviate from the stipulated figures. Legal authorization for the performance of such tests is only granted on condition that laboratories perform a successful external round robin test. They will receive a certificate which will be valid for a short time period, namely a couple of months. Such certificates warrant technical expertise to analyze certain substances such as medication, drugs, alcohol, and others; other certificates are granted for expertise in dealing with materials such as hairs, blood, urine etc.

This shows that quality assurance of the forensic-toxicological analyses which are performed on the occasion of an aircraft accident, is considered to be extremely relevant; Therefore it is indispensable to perform careful quality control.

Before the actual analyses are carried out, the pathologist who is responsible for the autopsy and takes the samples, and the toxicologist need to discuss the appropriate way of proceeding the samples. So, the pathologist will separate a piece from a central part of a muscle and change the scalpel frequently to provide uncontaminated material for investigation by the analyst. Contamination can be caused by POL, such as lubricants, Avgas or other chemical substances or pollution which can have affected the corpse at the crash site and which can interfere with the analysis of the materials. Moreover, continuous feedback is required during the course of analyses in order to enable the discussion of first test results so that their relevance can be evaluated in the individual case, even in the face of new questions and further examination methods to be applied. On the other side, feedback from the laboratory can trigger improvements in the sampling method, or call for taking necessary samples of different material.

3 FOCI OF EFFORTS IN TOXICOLOGY AND PATHOLOGY

3.1 Alcohol

The identification of alcohol in serum, blood, or urine samples taken from living probands is unproblematic, even after storage in the refrigerator at 4° C temperature for several months. The concentrations of congener alcohol in beverages can just as well be differentiated regarding type and quantities of alcohol consumption (wine, beer, spirits, etc.), even if the person involved alleges to have consumed massive portions of alcohol posterior to the accident.

However, examination of aircraft accident victims can be a difficult task when corpses are heavily disintegrated or were exposed to excessive heat of the sun or of flames; sometimes autolysis and putrefaction have set in or the remains are contaminated by POL substances or extraneous matter. The

laboratory tests aim at determining blood alcohol concentrations which prevailed at the moment of the crash.

When blood could be taken during the autopsy, it must have been properly extracted from the femoral vein, since it is located far enough from the stomach to exclude potential false results which could be caused by postmortal diffusion processes. Alterations of water content in the blood and in the body may be conducive to false results which should be corrected in accordance with the water content found in the test material. Simultaneous tests of congener alcohol such as propanol-1, methanol, propanol-2, butanol-1, and butanol-2, isobutanol, and 1 and 2- methylbutanol-1 etc. disclose the possibly postmortal formation of alcohol. The precondition is the evaluation and control of the presence of so-called putrefaction markers. However, if such putrefaction markers are increased, microbiological exams will be required additionally to detect the presence of bacteria and fungi. According to the type of microorganisms, very different congener alcohols can develop as metabolic products such as the above mentioned types of alcohol and amino acids. They are of decisive importance for the assessment of the blood alcohol concentration of the persons involved at the time of the accident.

In the presence of abdominal injuries, blood alcohol concentrations can be distorted, even if samples have been taken properly from the femoral vein. In such cases therefore, it is necessary to examine other tissues and liquids, such as cerebrospinal fluid, cerebral tissue, muscles from the extremities, vitreous fluid, bone marrow, lung and kidney tissue, urine, gastric contents, synovial fluid and others and to determine the water content. Aircraft accident investigation should pay special attention to hematomas for detection of alcohol, since hematomas keep the alcohol concentrations at the moment they occurred, namely at the time of the crash. The results of numerous and different materials provide for the determination of the distribution phase of ethanol (phase of resorption or of elimination). It is evident that - just as blood - all the other samples have to be tested for the existence of congener alcohols and microbiological test methods have additionally to be applied because the development of alcohol may have progressed at different speed in the various materials. In cases with severely putrefied samples usable results are often obtained from the test of the vitreous humour, because there bacterial degradation occurs only at a late stage.

3.1.1 Factors possibly affecting the blood alcohol content

Full blood is made up by corpuscular components and the blood plasma. Serum (blood plasma minus fibrinogen) has a water content of about 90%, as compared to full blood whose water content is about 80%. Alcohol concentration of the living is determined in blood serum obtained by centrifuging and the value is adjusted to full blood using the above mentioned water contents ratio. As for corpses this cannot be done. In this cases the alcohol concentration is calculated using the actual water content of cadaver blood and the average water content of full blood of 80 %.

This procedure can also be applied to the other materials to be analyzed. In analogous manner this applies to the calculation of the congener alcohols.

3.1.2 Formation of alcohol due to bacterial activity

In an environment of putrefaction, bacteria need a carbohydrate substrate like glucose to produce alcohol. Due to bacterial activities of various Proteus strains and other bacteria, blood alcohol levels up to 2 $^{o}/_{oo}$ and urine alcohol levels up to 5 $^{o}/_{oo}$ and more could be measured, depending on the respective glucose concentrations. A whole series of ubiquitous bacteria and fungi produces alcohol, such as: Escherichia coli species., Pseudomonas species, Pulularia species, Candida albicans and many more. Anaerobic and aerobic bacteria are distinguished from one another according to the different patterns of the above-mentioned congener alcohols they can produce in addition to ethyl alcohol; they differ from yeasts which as a rule do not produce such a great variety of substances. The formation of these bacterial decomposition

products is controlled by temperature, pH value, concentrations of available carbohydrates and other nutritive substances. On the crash site, this problem must be accounted for when dealing with aircraft accident victims, in order to avoid any mistakes when the true causes of a crash are looked for. The detected alcohol which has been taken in with beverages cannot be distinguished from alcohol which has been produced by bacterial activity. This is another reason why a large quantity of tissue and fluid samples which are not affected by putrefactive changes, should be safeguarded to enable correct interpretation.

Experience has shown that clostridia and a variety of Proteus bacteria produce the highest concentrations of ethanol. In addition to ethanol, several amino acids are produced according to a special fermentation pattern, such as α-aminobutyric acid and γ-aminobutyric acid, or δ-amino valeric acid. One has therefore to conclude that samples of material which show criteria of microbial formation of congener alcohols and amino acids must be excluded from the start to answer the question if alcohol has affected or not fitness for flying duty.

3.2 The question of chronic alcohol abuse

Whereas, in the case of acute alcohol intoxication, histological tests do not disclose any characteristic findings, in chronic alcohol abuse histological findings in the liver are dominating. It must be taken into consideration, however, that the various findings are unspecific by itself. Only the spectrum of morphological hepatic changes together with the findings of the clinical history (which were documented during the test for aeromedical disposition) permits the assessment if alcohol abuse is really causal for hepatic damage. Large-scaled test series disclosed that approximately 20 to 30% of chronical alcoholics had normal findings in liver biopsies. Alcoholic fatty livers show in enzyme-histochemical tests patches of deficient activity of the lactate dehydrogenase including stronger NADPH-dependent reactions of aldehyde. But independently of the fatty degeneration of liver and beyond, other degenerative and inflammatory alterations due to alcohol abuse are found in the liver, such as chronical or acutely inflammatory infiltrations of the portal fields and lobules; particularly in the area of necrobioses and individual necroses where central lobular sections are preferred. The true designation would be toxic hepatosis or fatty liver hepatitis in alcoholics. (In the anglo-american environment this finding is called “acute alcoholic hepatitis”.) Hyaline deposits within the cell plasm are very characteristic, particularly when they are found in the centrolobular hepatocytes which are denominated “alcoholic hyaline” or “Mallory bodies”. These are blurred cloudy cytoplasmic solidifications close to the nucleus which manifest themselves in plump or elongated form and acidophilic consistency. The electron microscope study shows the hyaline material to consist of drop-shaped or striped assemblies of moderate electron density; under a more powerful resolution, the three layer structure of the membranes become evident together with granular portions similar to ribosomes. This alcoholic hyaline must be clearly distinguished from giant mitochondria which are rarely found. These are small homogenous clearly defined cytoplasmic inclusions in the hepatocytes, the contour of which is plump or like a cigar. The last-mentioned finding is unspecific and can be interpreted as insufficiency of the mitochondria. In general, 40 % of the cases of fatty liver hepatitis are found with Mallory bodies. In most cases, the Kupffer star cells are enlarged and increased in number and often contain deposits of iron pigment which is called in German “drinkers’ iron”. In the central lobular areas is found an increase of reticular and collagenous fibers which seem to be woven in by a net of individual or by groups of hepatocyte epithelia. This picture of “wire-netting” fibrosis together with Mallory bodies is considered to be a powerful indication for the existence of chronic liver damage caused by alcohol abuse. The chronic course of fatty liver hepatitis is manifest by an increased number of histiocytic cell infiltrations and activated star cells. Going out from the lobular center, the hepatic parenchym will fibrose and sclerose at a later stage including the intermediary and periportal areas. The resulting hepatic cirrhoses have different manifestations: they are either throughout nodose or have differently sized parenchymous patches, the so-called post-necrotic cirrhosis which is found in 10 to 20% of all alcoholics. However, this condition presupposes a 10 to 15 years’ time period of development. Pancreatic inflammations due to alcohol intake are found in combination with hepatic alterations also due to alcohol abuse in a number of cases. More than one third of the cases of chronically sclerosed

pancreatitis are caused by alcoholism and this is the prototype of pancreatitis due to alcohol abuse. It is a very intensive peri- and intralobular sclerosing associated with parenchymatous atrophy and no relevant interstitial inflammatory infiltrations. Moreover, dilatations and thickened secretions are found in the glandular cavities of the acinus, ductal ectasias, epithelial flattening and pronounced parietal sclerosis of the pancreatic vessels. The islets of Langerhans often show moderate peri- and intrainsular fibroses. Lipolytic and proteolytic foci can just as well occur as inflammatory infiltrates or smaller hemorrhages.

Also the central nervous system discloses different histomorphologic findings which are caused by chronic alcohol abuse. The most important diseases initiated by alcohol include Wernicke's encephalopathy with alterations in the area of the 3rd and 4th ventricle and the cerebral aqueduct. Proliferations of the glia and particularly of the astroglia and the microglia are observed together with the vegetation of the capillaries whose walls are thickened. Seldom found and prevailing in the Mediterranean countries, where alcoholics prefer red wine, alterations of the brain are found as a pontine myelinosis or a primary degenerative disease of the cerebellar cortex preferably of the vermis and the alcoholic amblyopia which can have been preceded by visual disturbances.

Other alterations which - together with certain symptoms combinations - are indicative of the development of haemorrhageous internal pachymeningopathy extending over the frontal parts of the cerebrum and whose symptomatic is the thickening of the connective tissue of the pia mater.

Histological examination of cardiomyopathy as a consequence of chronical alcohol consumption as mentioned by clinical doctors shows - among other findings - hypertrophic and partly degenerative muscle fibers, various degrees of fibroses in the myocardium, blotchy endocardial fibro-elastoses, parietal thrombi, and inflammatory foci in the endocardium and epicardium. The electron microscopic examination proves the intumescence of the mitochondria reducing the size of the cristae. The myofibrilla reveal various changes even the complete loss of the striation and the dissolution of the myofilaments.

The histo-morphological findings just mentioned can only be considered to be a hint for a chronic alcohol abuse; however, when the laboratory parameters are evaluated and taken into account such findings become still more relevant. So, on the occasion of aircraft accidents, the records of the tests for aeromedical disposition, especially the laboratory parameters are looked into, not only for assessment of the consequences of chronical alcohol abuse, but also regarding other morphologic-pathological changes.

The medium corpuscular erythrocyte volume (MCV), gamma glutamyl transferase (GGT), the transaminases ALT and AST and their ratio, the methanol concentration, and CDT (carbo-hydrate deficient transferrin) are considered to be the typical biochemical markers for alcoholism. CDT determination has shown that commercial kits are inappropriate for assessment but it is necessary to carry out this test by high pressure liquid chromatography (HPLC). Based on a study of our pilots collective, we assume the normal value to be up to 2 % of Disialo-transferrin. The decisive advantages of the HPLC method are as follows: complete separation of the alcohol-relevant isoforms of transferrin and avoidance of false-positive results.

There are three genetic principal types B, C, and D of the iron transport protein Transferrin which again can be divided into more than 20 sub-types. The C-type prevails by 90% within the population. As far as known by now genetic variants are due to a point mutation in the DNA sequence. The result of this condition is a change of the amino acid sequence of the protein and this entails a change of biochemical qualities. According to the method of CDT determination, certain genetic constellations are likely to produce false results. So, the CDT-determination of a C/D heterozygous person will produce a false-positive result, whereas the analysis of a B/C heterozygous person will render too low values, if one of the common immunoassay methods are applied. Due to the principle of the method such false values are not even obvious. Such false results can only be avoided, if the HPLC method (possibly with iso-electric

focussing) is used for CDT-determination, since the HPLC chromatogram provides for detecting the B/C or C/D variants.

3.3 On the effects of POL substances and combustion gases

Aircraft carry large quantities of fuel onboard; in addition there are deicing agents (containing frequently alcohol), lubricants, hydraulic oils, hydrazine, and other liquids. In an aircraft crash, the fuel - mostly F-34 - immediately catches fire due to its high ignitability; as a result large quantities of combustion gases develop in no time. On the one hand, such gases can be aspired by the surviving victims, on the other hand they can affect postmortal investigation considerably since they have contaminant properties. Therefore, it is sometimes very difficult to answer the question, if the fire conflagrated during flight with the consequent development of combustion gases, or if the fire broke out at the moment of the crash. Moreover, plastic material which is built in the aircraft can degrade to hydrochloric acid (HCl) and finally prussic acid (HCN) due to contamination. In the autopsies, we try to avoid such problems when the corpses are contaminated by the choice of the investigation material. So, when large quantities of fuel or other POL have poured over the corpses and have diffused through the skin due to their high fat-solubility we often change the scalpel and after removing superficial layers of the femoral muscles we take off deep muscular tissue. Suitable is also bone marrow or liquid from the larger joints. Kerosene and other contaminating substances preclude evaluation of the material by chromatography techniques; under such circumstances the questions we are interested in cannot be answered.

Contaminations, however, can be caused by other conditions which I would like to describe in the following:

During a training flight in Canada, 150 NM south-south-east of the Goose Bay airfield, a Tornado PA 200 crashed after having touched ground in a narrow valley. The crew lost their lives. Both crew members died as a consequence of polytrauma combined with craniocerebral trauma, massive thoracic and abdominal injuries associated with excessive loss of blood. According to the toxicological investigations, neither the pilot nor the weapon systems officer (WSO) had been influenced by alcohol, drugs or medication. The toxicological examination (GC-MS) of blood samples taken from the pectoral cavity of the WSO disclosed the presence of volatile substances such as intensive to very intensive signals of α-pinene and β-pinene, lemonene, and β-phellandrene, and small to very small signals of camphene, β-mycene, 2- and 3-carene and α-phellandrene.

Some herbal drugs against the common cold, such as Gelomyrtol® contain such and other similar volatile substances. The medical records of this pilot did not disclose any clue regarding the intake of such a drug. All such volatile substances however are the typical components of pine and cone oils such as of most coniferous trees. When the autopsy reports were consulted, the victim was found with several wood particles in the right upper abdomen which had penetrated him from the transitional part of the right part of the neck towards the shoulder and perforated the pectoral cavity and the diaphragm and which had thus contaminated the material to be analyzed in our laboratory. The pine needles which were safeguarded at the crash site were analyzed by gas chromatography mass spectrometry procedures proved to have the same volatile substances of comparable intensity.

If no contamination occurred, as a rule it is not difficult to prove the existence of inhaled combustion gases. F-34 fuel is a mixture of saturated hydrocarbons (alcane and cycloalcane), unsaturated hydrocarbons (alcenes and aromatous compounds) such as benzene, toluene, and naphthalene and technically caused pollution such as sulfides and disulfides, sulfurous hydrocarbons such as mercapane and nitrogenous compounds (pyridines and homologous substances). Moreover, there are additive substances which have to be considered such as icing inhibitors (ethyleneglycol monomethyl ester), corrosion inhibitors, anti-oxydant agents, agents against static charge, and substances for the improvement of flow properties. The most important component of all the other POL substances such as lubricants and

hydraulic oils also contain fractions of mineral oil or synthetic oils. In the toxicological investigation practise, additive substances are relevant only regarding the identification of fuel. Pyrolysis and oxidation products which developed during the combustion of hydrocarbons are of exclusive relevance. Sometimes, the identification of carbonmonoxide proves to be difficult with degraded bodies, since the common photometric methods do not yield reliable and substantial results. Since, under deficient oxygen supply and very high combustion temperature, combustion remains incomplete, the result will be carbonmonoxide making up a very strong compound with hemoglobine or myoglobine which will have the well-known consequences.

Our Division Forensic Medicine and Aircraft Accident Investigation applies as a routine photometric procedure the measuring of nine important wavelengths. Due to the difficulties arising with photometric methods which seem to be applied in all forensic medical institutes in Germany, in addition to this photometric method we developed the analytic method of gas chromatography which is not or not more used in other institutions, because it requires excessive efforts. Gas chromatographic separation is performed on columns packed with molecular sieves or capillary columns. Detection is achieved with a flame ionization detector after reduction of CO to methane by hydrogen. Samples for analysis are muscles or the inner organs, when no blood is available due to disintegration of bodies or the bodies are found in advanced state of autolysis and putrefaction. Carbon monoxide bound to myoglobin or to the hemoglobin in organs is measured in such cases. Sometimes the determination of cyanide is problematic too. In the first place we practice gas chromatographic separation on packed or capillary columns of the volatile hydrogen cyanide and photometric detection.

Due to the normally very scarce concentrations of substances whose existence is to be proved (POL material, pyrolysis and combustion products), such substances must be concentrated and interfering matrix components must be removed from them. For this purpose, Headspace Techniques, Purge&Trap Systems, and Headspace Solid Phase microextractions are applied in the first place.

3.4 Postmortal diagnostics of disturbances of the glucose metabolism

3.4.1 Pathological findings

When the causes of an aircraft accident are investigated, the possibility of disturbances of the glucose metabolism must be taken into account. The diagnosis of diabetes mellitus or the diabetic coma are of particular relevance in an aircraft accident investigation. Apart from the findings of the internal and external inspection of the body, namely the autopsy which discloses - among other things – punctures and epidermal findings typical for diabetes like chronic ulcers or epidermatomycosis and are associated with obesity or a poor nutritional condition, the following findings are indicative for the above-mentioned condition: the stiff condition of the brain substance, xanthochromia of the cranial vault and of the subcutaneous fat tissue as well as several varieties of chronic pancreatitis and renal alterations like the Kimmelstiel-Wilson glomerulosclerosis. The following phenomena can be indicative of the presence of a diabetic coma: cerebral edema, acute terminal pancreatitis, swollen pale kidneys in the presence of glycogen nephrosis with large phytoid Armanni-Ebstein-cells in the straight areas of the proximal tubule in the lower part of the medullary rays and in the external medullary zone. Histologic signs associated with persistent diabetes mellitus are a general microangiopathy, typical annular nuclei of hepatocytes, island hyalinoses, island amyloidoses, and island fibroses of the pancreas. Histochemical tests can prove reduced zinc contents accompanied by a fibrosis of the exocrine pancreas. B-cell reduction of the Langerhans-islets is a specific indicator of the existence of juvenile diabetes Type I, associated with nodular glomerulosclerosis (Kimmelstiel-Wilson).

3.4.2 Biochemical findings

As a supplement to histomorphological findings it is necessary to conduct biochemical examinations when a diabetic coma or a fatal hypoglycaemia is suspected. Biochemical analysis can provide the best clues,

including the determination of insulin or measuring C-peptide. Postmortal biochemical analyses require body fluids to be taken such as cerebrospinal fluid, vitreous humour, blood, and urine. The following parameters must be determined: glucose, lactate, HbA_{1c} , ketone bodies, insulin and C-peptide. The inversely exponential glucose decomposition of cerebrospinal fluid is about 10 to 15 mg/dl per hour which means that it takes normally 10 to 12 hours until no glucose is remaining. If the existence of glucose can be proved after this time period, the existence of premortal hyperglycemia must be assumed. However, the evaluation of liquor glucose figures ranging normally between 50 and 90 mg/dl requires extraordinary care, because increases of the normal liquor glucose percentages can also be caused by other mechanisms such as CO-intoxication, trauma of the CNS, protracted agony, and others. Lactate concentration in cerebrospinal fluid is normally about 9 mg/dl; however, due to glycolysis in the post mortal phase there is an increase of about 10 to 15 mg/dl per hour until the tenth hour post mortem. Particular caution is required also for the interpretation of the lactate figures. We apply a combined method according to Traub and sum up lactate and glucose. Our calculation is based on the principle that one mole glucose produces 2 moles of lactate. The straight addition of the measured values can be performed when they are taken in the unit “mg/dl”. We consider 362 mg/dl to be the upper limit of the normal additional value in the cerebrospinal fluid. Any figures exceeding this value disclose metabolic imbalance, but they must be evaluated very critically. The same method using the total of glucose and lactate is also applied to vitreous humour.

Determination of glucose and lactate in blood did not prove to be useful, if blood of the right ventricle is used. The figures might be increased due to the hepatic glycogenolysis. Normally, however, postmortal glycolysis reduces within six to eight hours glucose in the blood to 0 mg/dl. Postmortal diffusion of serum and substrate from the tissues into the blood are so unpredictable that the application of the empirical formula according to Traub is not possible.

Another important parameter is the determination of hemoglobin A_{IC} (Hb A_{IC}). In this case a molecule of glucose is non-enzymatically added to the Hb-molecule (glycolized hemoglobin). As for the diagnosis of comatous condition , increased totals according to Traub, urinary glucose and Hb A_{IC} are applicable. Since Hb A_{IC} is relatively resistant to autolysis, its postmortal existence continues to be provable in frozen condition or when stored at +4°C temperature, for a considerable time period. Hb A_{IC} figures exceeding 12% are considered to be an indicator.

Moreover, we refer to the determination of ketone bodies such as acetone, acetoacetate and ß-hydroxybutyrate whose concentrations are high in the presence of extraordinary ketotic metabolic disorder. As a rule, tracing of free acetone is performed by gas chromatography procedures together with blood alcohol determination; when acetone values exceed 5 mg/dl, the existence of diabetes must be considered.

As for the unrine anaylsis, glucose concentrations exceeding 25mg/dl are an indicator for diabetes, however the possibility must be considered that the glucose increase mght have been conditioned by other circumstances such as cerebral trauma. Considering the ketone bodies, an increase of free acetone superior to 0.5 mg/dl is an indicator of the existence of a ketotic metabolic disorder. Diagnosis of hypoglycaemias is a considerable problem. They are divided into exogenous and endogenous hypoglycaemia. Exogenous hypoglycaemia is triggered by erroneous or intentional administration of insulin or sulfonylurea. Endogenous hypoglycaemias are observed, among others, in the presence of diseases such as insulinoma or when a person abstains from food after excessive alcohol consumption.

According to Traub, low totals detected in liquour (inferior to 50 mg/dl and in the vitreous humour inferior to 100 mg/dl) with simultaneous high insulin concentrations indicate the existence of hypoglycaemia. We would like to draw your attention to the fact that the intake of sulfonylurea will trigger an increase of insulin and C-peptide.

3.5 Particularities as to the microsopic examinations of the heart

Considering the eminent importance of the question if cardiac failure – e.g. of inflammatory origin - could have caused the accident, the hearts of crew members have been examined by special methods for many years. If for the assessment of the cardiac condition only a few sections are performed at the left and at the right-hand side of the heart, this assessment applies just to the area which was examined. Since inflammatory processes can occur at any point at different cardiac regions - we have decided a couple of years ago to take samples from 19 places of the heart and to provide complete section and evaluation of the coronaries in each and every aircraft crash victim. The places for sampling are chosen in accordance with DOERR's examinations regarding the distribution pattern of infiltrates which occur during myocarditis of different causes. So, we have gained experience with more than 100 cases in the course of the passed eight years.

I would like to show you three cases:

When approaching his main operating base, an antiarmor helicopter suddenly took to a steep descent and crashed into a meadow. Technical investigation found out that this attitude could only have been caused by sudden lowering of the so-called collective pitch lever. When the autopsy was performed, macroscopically no essential pathological findings were achieved regarding the organs. In order to rule out a sudden conductive disturbance, the whole ventricular septum was investigated in about 2000 step sections which exceeded the normal scheme of sampling. The septum disclosed several small lymphocytic infiltrations outside of the pathways without any secure signs of muscle cell necroses which could be followed for about half a millimeter. According to the DALLAS criteria this finding corresponds to an idiopathic borderline myocarditis. Additionally, lymphocytes and plasma cells proved to be accumulated on various places in the lymphatics; the former can be interpreted as drainage of various foci of inflammation which could be proved to exist. In the evaluation however, we considered it rather improbable that the airworthiness should be hampered by such findings, since the distance of the infiltrations was too far from the pathways and also taking slightly distinct clinical cardiovascular features into account.

The second case is as follows: A Tornado fighter jet pilot performed 15 attacks to a ground target with low acceleration forces. In the approaches 16 and 17, acceleration forces increased to 6 G which means that the gravitational acceleration was sixfold. In his 17th approach the pilot initiated the inception turn too late and crashed against a bunker building.

Macroscopical examinations of the organs did not yield any essential pathological findings. Histological examination revealed multiple netlike fibroses of the myocardium and medial and intimal hypertrophy of the arterioles. In some sections, the Luxol-Fast-Blue staining showed the characteristics of diffuse myofibrillar degeneration. Several areas were found, where cardiac muscle fibers in wavy course bordered on distinctly fragmented myocyte bundles. Of late, this change is interpreted to be an indication of a beginning myocardial damage.

Attending to the clinically recorded distinctive features - particularly in the exercise electrocardiogram and from the aeromedical point of view - an acute functional impairment of the myocardium under the sudden excessive G-loads cannot be ruled out to have been the cause of the aircraft crash.

The third case: This case is supposed to demonstrate that extensive cardiac investigation is also rewarding in other circumstances.

Under the microscope, the myocardium of a patient who had suffered from tuberculosis showed several tubercles which were located at the right posterior auricle and at the left posterior ventricular wall close to the base; such tubercles explained sudden death although pulmonary findings had improved. Molecular

genetic investigation proved bacterial genome to exist in those areas and thus a rare case of tuberculous myocarditis could be verified according to the pertinent literature.

Since pathological changes of the myocardium can occur at diverse places and since foci of inflammatory alterations close to the conduction pathways - in particular - can trigger disturbances of spread of stimulus, the interpretation of just a few histological sections performed at only one place of the right and the left ventricular wall do not prove very much. Therefore, extraordinary expenses and efforts are required and a large number particularly of critical regions of the myocardium need to be investigated.

4 FINAL REMARKS

Apart from the problem areas which were mentioned regarding the toxicological and pathological examinations, diverse other fields would have to be discussed critically, such as interpretation of vital reactions, appreciation of the time, when injuries were inflicted, ability to act after having suffered different types of trauma, and the evaluation of toxicological tests which were performed with hairs, or the differentiation between real drug consumption and having eaten a piece of poppy seed cake, for example.

Only if all aircraft accidents and incidents are continuously examined subtly and in every detail, attending to a very high investigation standard, a high degree of flight safety can be achieved. This means that the examinations which must necessarily be performed for securing the evidence and clarification, if the accident could have been avoided, exceed by far the extent of the investigation which would be required for criminal appraisal only.