UNIVERSITY OF GLASGOW

DROWNING : A STUDY OF TEMPORAL BONE HAEMORRHAGE AND AN ANALYSIS OF VITREOUS HUMOUR

A THESIS SUBMITTED FOR THE DEGREE OF MASTER OF MEDICAL SCIENCE

BY

EMAD ABASS ABOOD ALHEMYARI

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DEPARTMENT OF FORENSIC MEDICINE AND SCIENCE,

THE UNIVERSITY OF GLASGOW

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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C O N T E N T S

3.3. Swimming pool drowning

4. Discussion

4.1. External examination

4.2. Internal examination

4.2.1. The froth

4.2.2. Voluminous lung

4.2.3. Water in stomach

4.2.4. Cardiac dilation

4.2.5. Fluidity of the blood

4.2.6. Petechial haemorrhage

4.3. Swimming pool drowning

4.4. Bathtub drowning

4.5. Alcohol and other drugs

4.6. Immersion in cold water.

4.7. Other pathological changes

5. Conclusion

SECTION 1: Histological examination of the lung tissue in drowning and other asphyxial cases

1. Introduction

2. Material and methods

3. Results

3.1. Drowning death

3.2. Cardiac cases

3.3. Cases of brain injury

3.4. Mechanical injury

3.5. Cases with inhalation of gastric content

3.6. Miscellaneous cases

3.7. Drug and gas poisoning

2.2. Fixation

2.3. Sawing

2.4. Decalcification

2.4.1. Initial decalcification

2.4.2. E.D.T.A.

2.4.3. Formic acid citrate

2.4.4. 10% Formic acid

2.4.5. R.D.C.

2.5. Washing

2.6. Processing

2.7. Vacuum embedding

2.8. Embedding

2.9. Sectioning

2.9.1. Microtomes and knives

2.9.2. Frozen section

2.10. Staining

3. Results

3.1. Typical drowning

3.1.1. Intracavitary haemorrhage

3.1.2. Mucosal lining of tympanomastoid spaces

3.1.3. Inner ear examination

3.2. Immersion syndrome

3.3. Other cases recovered from water

3.4. Bathtub drowning

3.5. Hanging

3.5.1. Intracavitary haemorrhage

3.6. Asphyxia

3.7. Intracranial haemorrhage

SUMMARY :

Deaths by drowning are a regular occurrence in the practice of forensic medicine.

Since the external and internal signs are not always classical, the diagnosis is often determined by the exclusion of other causes of death. Some of the signs are regarded only as supportive evidence of drowning and cannot be used to establish accurately if the victim was breathing at the time of his immersion. Additional techniques have therefore been developed in attempt to establish more definitely, a diagnosis of drowning.

Examination of internal organs for minute unicellular algae called diatoms is one such method and it has been successfully achieved by using an enzyme digestion method. One of the most significant aspects of the diatom test is the examination of the lungs and the distribution and number of diatoms in them. However, the value of the diatom test as a diagnostic method is still not entirely decided; it merely confirms that absorption of diatom-containing media has taken place. Therefore, while the diatom test cannot be considered as conclusive evidence of drowning, it has resolved some difficulties in the diagnosis. It is not considered a final solution to the problem.

Drowning is a more complicated mechanism than mere inhalation of water and the results obtained in this work by the diatom test support this.

Temporal bones from cases of drowning, possible drowning, other forms of asphyxia, and miscellaneous causes of death were examined to determine the reliability of intrapyramidal haemorrhage as a distinctive sign of drowning. Intracavitary haemorrhage of the middle ear and mastoid air cells is a highly characteristic feature of drowning; submucosal haemorrhage of the lining mucosa of the air cells is, also, an important sign and is considered a noteworthy feature. Although intracavitary and submucosal haemorrhages were detected in cases other than drowning, they were of a milder degree and grade than those found in true drowning. By using this finding, i.e. intrapyramidal haemorrhage, it has proved possible to establish or exclude a diagnosis of drowning. Moreover the lesion is a vital phenomenon, and by excluding other conditions, it can establish whether the victim was alive or dead before immersion.

To estimate the immersion time in cases of drowning, the vitreous humour was analysed for Magnesium, Calcium, Potassium, Sodium, and Chloride to find a suitable correlation with the time spent in the water. It appears that dilution of these elements in fresh water submersion is caused both by water entering the hyperosmolar vitreous humour and ions diffusing out of it. However, individual variation in the degree of dilution restricts the application of the method in estimating the immersion time. Though there was an obvious depletion of vitreous

humour ions, it was not consistently related to the time of immersion. It appears, however, that sodium and chloride in fresh water cases are related, albeit erratically, to the length of the immersion period.

CHAPTER 1

DROWNING: POST-MORTEM DIAGNOSIS AND CRITICAL ASSESSMENT

1. INTRODUCTION AND LITERATURE REVIEW:

1.1 DEFINITION:

Webster's dictionary defines drowning as to suffocate by immersion specially in water. Others used the term, asphyxia (110), instead of suffocation as it is caused by defective oxygenation of blood in the lung due to the presence of fluid in the respiratory tract which enters through the nostril and mouth.

The term submersion is used indiscriminately in most of the definitions (66) while other authors are more precise to use "partial submersion" as a possible occurrence in cases of drowning (61,110,184). This controversy in definition is partially due to the difference in determining the cause of death in drowning. Some believe that the cause of death is due to complex pathophysiological changes (140) rather than just an asphyxia (61,66,110) or circulatory arrest (184). Therefore, some researchers denied an over simplification of definition as being just an asphyxial death due to submersion in water (65,103).

For lack of the most satisfactory definition, some agreed to choose either one of two mechanisms,

i.e.asphyxia or biochemical changes of blood as causes of death due to submersion in fluid (184), while others preferred the most simple definition as suffocation from submersion in liquid (112). None of these is completely satisfactory (107).

Generally, the definition of drowning allows liberal use of the term to cover the rarer episodes of submersion in quick sand, oil tanks, vat of malmsey fermentation tanks, bitumn tanks, vat of beer, gasoline and paint as well as submersion in the usual media such as rivers, lakes, seas, well tanks, ponds, canals, swimming pools and bathtubs.

The meaning of fluid or liquid in cases of drowning does not apply to the stomach contents (61) which causes a totally different mechanism of death.

1.2. TERMS USED IN CONNECTION WITH DROWNING:

There are general terms which have been used to implicate drowning as an indirect cause of death in bodies recovered from water. One of these terms is secondary drowning which is defined as state of deterioration which occurs without an original respiratory paralysis when the patient is rescued from water immediately but has meanwhile inhaled water (61,134,186). It may occur after the victim has been successfully revived or at least survived for a spell after rescue (186). However other terms have been used to indicate the condition of a patient who dies from

respiratory deterioration after being rescued from water (186), e.g. post-immersion death (63), secondary drowning (130) and near drowning (186).

Post-immersion death, for example, has been described in shipwreck survivors (63), and is thought to be caused by "after-drop phenomenon" of temperature (20) as a leading cause of ventricular fibrillation in these cases (92). Hypoxia and pulmonary insult are emphasized as being the the primary factors in cases of near-drowning (56). Death in these cases is due to acute pulmonary oedema which might occur at any time from 15 minutes up to 72 hours after immersion (63). Secondary drowning should be reserved for patients who are asymptomatic and without any laboratory abnormalities, but after a latent period they develope symptoms without evidence of intervening progressive respiratory insufficincy (134). It is reported to occur in approximately 5% of submersion deaths (134).

1.3. OCCURRENCE:

1.3.1. ACCIDENTAL DROWNING:

Accidental drowning is probably the commonest type (61,96,132,146,148). It occurs usually in localities when a pathway runs close beside the bank of a canal or a river. The victim is usually male, or a child. Usually signs of violence are absent and circumstantial evidence strongly suggests an accidental death, especially if classical signs of drowning are present. Distribution of

accidental drowning by sex and age is similar in most of the studies and statistics (1,26,34,119).

Drowning is held to be the fourth leading cause of accidental death in the United States (186) which accounts for up to 8,000 deaths each year. The total figure is estimated to be about 150,000 drownings/year in the entire world (35), with a rate of 6 fatal submersion /year/100,000 population at risk which accounts for 17,616 years of lost productive life (33). The rate of drowning was reported to be 5.6/100,000 in Australia, 4.6/100,000 in U.S.A. and 4/100,000 in the U.K. (109). The crude fatality number in the U.K. is about 1500 p.a. with 75% occurring in inland water i.e. fresh water (110).

There are a few typical situations leading to accidental drowning which are mentioned here briefly to be followed by a more wider discussion later. One of these situations is swimming pool drowning which is a common type of accidental drowning among children (33). Sustained injuries are not uncommon, especially a fracture of the cervical spine with injuries to the spinal cord owing to the hitting of the head at the bottom of the pool following the jumping into shallow water (146). Another type of drowning in the swimming pool is called hyperventilation death (61,63). The term is derived from the act of hyperventilation prior to jumping into water. There is another type of drowning

which occurs during immersion in cold water which is caused by uncontrollable hyperventilation due to the sudden cooling of the skin by immersion in cold water (90,91,92). Such a reaction is thought to be strong while a person is under the influence of alcohol which is quite a common phenomenon related to drowning (34).

Accidental death due to scuba diving is the most frequent cause of death among amateur divers (61). Hazards of scuba diving which lead to death by drowning are asphyxiation, arterial gas embolism, head injury, heart attack, aspiration, decompression sickness. A further group of accidental drowning results from natural and unnatural causes with ultimate inhalation of water. Various natural causes have been held to account for drowning, such as epilepsy, mental retardation, heart diseases, Parkinson's disease, osteoarthritis, Friedrich's ataxia, ruptured spleen and acute haemorrhage due to rupture of large splenic cyst (33,34,61,65,148).

1.3.2. SUICIDAL DROWNING:

Drowning is a common method of self-destruction (61,65,96,132,146,148), chiefly among women, with special preference to immersion in a bathtub (69).

The ingestion of alcohol and/ or barbiturate drugs etc., is usually encountered as a conscious-weakening measure before drowning in the bathtub (62). Sometimes the diagnosis of suicidal drowning is straightforward indicated by circumstantial evidence such as the presence

of a suicide note, a past history of threatening to commit suicide. The victim may bind himself, attach weights to his body or inflict simultaneous injury on himself (61,69). Drowning may be achieved in very shallow water, or even by putting the head in a pail or cistern without the necessity for the body to be fully submerged (61,69).

1.3.3. HOMICIDAL DROWNING:

This form of drowning is rare (65,132,146,148). Although it is not easy to drown a healthy and conscious person forcibly, it may occur in children and helpless people, and should not be overlooked as a cause of frank child abuse (62).

It was cited in Egypt, for example, that 1 out of 20 bodies recovered from water had showed evidence of murder before immersion (148). The injection of insulin to render the victim unconscious before being forcibly drowned in a bathtub has been reported to be one notorious way of drowning (132). There are, also, famous cases of homicidal drowning (62) where the victims were murdered so successfully without leaving any sign leading to the suspicion of foul play.

1.4. PROCESS OF DROWNING:

The process passed through in drowning has been divided experimentally into 5 stages, these are; a stage of surprise, a stage of respiratory arrest, a stage of deep

respiration, a second stage of respiratory arrest and a stage of terminal gasp. These stages usually last for 5-10 seconds, 1 minute, 1 minute, 1 minute and 30 seconds respectively (17).

The first stage is well known to all swimmers who jump into cold water with resulting hyperventilation as a result of a reflex activated through the irritation of the receptors of the skin by unexpectedly cold water (91). There are two chief forces involved in submersion of a body at the time of the fall into water, these are the force of the fall and the specific gravity of the body itself. Usually the victim rises to the surface owing to the natural buoyancy of the body, and his irregular struggle movements to try to save himself. Inhalation and swallowing of a certain amount of water are made on reaching the surface . Inhalation of water stimulates spasm of the larynx and involuntary coughing which lead to further dyspnoea. It is thought that at this stage, 10-15% of immersed people expire because of reflex cardiac inhibition or mechanical asphyxia (184). Soon, exhaustion supervenes and the body sinks permanently with loss of consciousness. Convulsive movements then occur followed by a stage of terminal asphyxia in which automatic respiratory efforts cause water to be drawn into the respiratory passages. Eventually lethal fluid exchange across alveolar wall (17) will occur depending on tonicity and ionic

composition of the fluid.

1.5. FRESH AND SALT WATER DROWNING:

Most of the biochemical, physiological and clinical studies on fresh and salt water drowning have been carried out on experimental animals (41,56,112,113,150,163), to discover the real mechanism of death in both types of drowning and which may produce different clinical pictures (110).

It has been claimed that the clinical and biochemical findings in animals may not be the same as in man. However most of the results obtained from near-drowning cases did confirm many features in man as well as experimental animals (56,113,163). In brackish water, for example, which may be isotonic with blood one may infer that exchange of water between alveoli and blood must be very limited.

In both, fresh and salt water drowning, the most consistent finding is acute asphyxia with persistent arterial hypoxaemia (112,163) and subsequent circulatory failure. Circulatory failure means that the circulation becomes so weak that administration of oxygen fails to resuscitate the dying subject (163).

1.5.1. FRESH WATER DROWNING:

It is well-recognized that drowning is due to inhalation of water into the air passages and lungs where most of the absorption of aspirated fluid takes place, by

transportation of the fluid across the alveolo-capillary interface (153,162,163).

Evidence from experiments on animals showed that drowning in fresh water resulted in massive absorption of fluid and dilution of the blood causing haemolysis with decrease of electrolytes to fatal levels with relative increase of potassium ions (41,110,162,163). This predicts acute ventricular fibrillation (44,55,60,110,153,162,163).

1.5.1.1. DILUTION AND HAEMOLYSIS:

It has been confirmed experimentally that dilution of blood occurs in fresh water drowning by an amount of water equivalent to 60-150% of blood volume which enters the circulation in a few minutes (163). The dilution of the blood is extreme and a very rapid mechanism so much so that in 3 minutes, 50% of the circulating fluid will be drowning fluid. For such precise estimation of the degree of dilution , the deuterium oxide method was used (163). The amount of aspirated fluid has been measured to know precisely the degree of dilution which was found to be 3% of blood volume for each 2.2 ml./kg body weight (112), with a wide range of dilution from 0.12-1.2 volume of water/ 1 volume of blood (73). Perhaps surprisingly, some authors do not share the idea of occurrence of haemo-dilution in fresh water drowning, as the data obtained from studies on human fresh water drowning and near-drowning patients do not support the hypothesis of

haemodilution (153,163). However haemoglobinuria was found in fresh water drowning which could not be explained by any mechanism other than intra-vascular haemolysis (135). Haemolysis causes liberation of potassium ions into the circulation. This has been confirmed by intravascular injection of distilled water into men and dogs with resulting haemoglobinaemia and haemoglobinuria (135). Intra-vascular haemolytic crises were found in fresh water drowning cases, sometimes to a remarkable degree (41). Some workers, however, claimed that haemolysis is equally severe in drowning as well as in non-drowning cases, as the values of haematocrit, plasma protein, potassium and specific gravity in drowning cases showed little difference from the values of those who died from causes other than drowning (153). Generally, the blood picture in fresh water drowning will show haemodilution, hypervolaemia, hypoproteinemia, hyponatremia, hypochloraemia, haemolysis and hyperkalaemia.

1.5.1.2. VENTRICULAR FIBRILLATION:

Ventricular fibrillation is a term used when the ventricles display rapid, ineffective and uncoordinated movements that produce no pulse and the electro-cardiograph has broad, bizarre and irregular complexes. It was reported in drowning a long time ago (8) and mainly from experimental studies in fresh water drowning.

It is thought to occur after total immersion in water within 1-2 minutes (44) or 2-3 minutes (110). Although there are doubts about its occurrence in fresh water drowning (163) the presumption is strong that it does indeed take place (44,163).

The mechanism that incites V.F. in fresh water drowning is not fully understood (162). Hypervolaemia, for example, has been incriminated as being the sole cause (8). Other causes, like disastrous electrolyte changes superimposed on hypoxia were claimed to cause V.F. (162). Other workers, however, put hypervolaemia along with haemolysis, gross electrolytes changes and severe anoxia collectively as possible causes of V.F. The electrolyte changes, in this concept, refer mainly to high levels of potassium (44,110) as being responsible for initiating the V.F. Potassium liberation from the intracellular compartment into the extracellular compartment is due to anoxia as well as intravascular haemolysis (44,110,162). Within the ventricular cells the concentration of potassium and sodium ions are 150 and 10 mmol./l. respectively, while in extracellular fluid the concentrations are 4.0 mmol/l. for potassium and 140 mmol/l. for sodium. Electrical disequlibrium in the heart leads to arrythmia, particularly when the extracellular potassium reaches a high level (up to 8 mmol./l.) due to leakage of the ion out of the cells as happens in haemolysis. Accordingly V.F. in fresh water drowning is thought to be so called potassium-fibrillation (183), as

electrocardiography showed similar changes to that of potassium poisoning.

V.F. is dose related (64). its occurrence is related to the weight of the animal and the amount of fluid aspirated (64,112). There will be no fibrillation when the animal does not aspirate much water or it is unlikely to occur if less than 10 millilitre of fluid is aspirated per. pound of body weight (113). The general rule is that the larger the heart the more prone it is to permanent V.F.(163). That is why the larger animals develope V. F.readily, man is included in this category.

Whether V.F.is a characteristic feature of fresh water drowning or is more likely to occur in immersion which produces hypothermia is still without satisfactory explanation (88).

Work was directed to study the pathological changes in the heart in drowning to explain the rhythm disorders by investigating the role of catecholamines as causative agents for such changes (88). It is found that the type of focal and diffusely distributed lesions in cases of drowning was similar to those of catecholamine effects (165). The lesions include myocyte hypercontraction and hypereosinophilia (88). These findings are consistent with those which been found in phaeochromocytoma (165) and in an animal treated with catecholamines. Also the massive release of catecholamines is sufficient to cause severe myocardial necrosis, has been demonstrated in cases of near-drowning (88).

1.5.2. SALT WATER:

Salt water is markedly hypertonic (3.5%) compared to blood (0.9%). It is considered twice as lethal volume for volume as fresh water (112). Due to its hypertonicity it alters the lung parenchyma biochemically (113). As salt water enters the lung the ions pass from the alveoli into the circulation and at the same time there is a protein shift from the circulation into the alveoli (61,64,110,112,135,143,162,163) with resultant haemoconcentration, but without haemolysis or myocardial ischaemia (64,112,162).

Mechanical asphyxia, due to aspiration of fluid causes persistent arterial hypoxaemia, caused partly by the washing out of pulmonary surfactant, and thus increasing the load on the heart, slowing of the pulse, a fall in blood pressure and death (109,110), at the same time there is a rapid onset of pulmonary oedema (61,109,135,143,163). Death usually happen within 4-5 minutes (109), 8 minutes (132) or even up to 10 minutes (148), depending on the time of submersion and the amount of fluid inhaled.

1.6. ATYPICAL DROWNING:

In the vast majority of cases death by drowning in either fresh or salt water fluid should be considered separately (109,110), as it depends on the chemical composition of the submersion fluid. However it is not of

much importance whether the drowning fluid was fresh or salt but what does matter and more important is whether water is inhaled or not, as two different mechanisms of death have been recognized one with inhalation of water and other without inhalation of water (23,151).

Obstructive asphyxia due to larngyeal spasm is considered as one type of what is called dry drowning (61,103,132,173,184). Around 10-15% of drowning victims usually die of obstructive asphyxia before water enters the lungs in quantity, especially in a person whose laryngeal reflexes are brisk. It occurs as a protective mechanism through excitation of the pharngeal wall by inhaled water (184). The spasm of the laryngeal muscles is maintained by sealing the air passages until cerebral anoxia ensues with loss of consciousness and death without inhalation of water (110). Like other types of drowning the primary disturbance is acute asphyxia with persistent arterial hypoxaemia and acidosis. However, a lack of the so called classical signs of inhalation of water is the only difference between dry drowning as in cases of obstructive asphyxia and cases of classical drowning i.e. wet drowning.

Death after immediate immersion into water which occurs suddenly and sometimes instantaneous is a variety of dry drowning as a result of cardiac arrest (61,96,132,146,148,184). There are many terms which have been used to express this mechanism of death, such as vaso-vagal shock, vagal inhibition, reflex cardiac

inhibition and reflex cardiac arrest. Sudden death on immersion, clinically, is called immersion syndrome or hydrocution (96,132) in which neither mechanical asphyxia nor absorption of fluid medium cause death. It is regarded as a reflex inhibition of the heart mediated through the vagus nerve.

1.7. CHEMICAL DIAGNOSIS OF DROWNING:

Various physical and chemical methods have been used in order to find the most acceptable and reliable diagnosis of drowning. These methods included the examination of the followings; chloride, specific gravity, sodium, potassium, haemoglobin, plasma protein, magnesium, enzymes, osmolarity and red cells count

In most of these methods, attempts have been made to find a drowning test on the basis of a rule that the chemical and physical changes of blood depend upon exchange of water and electrolytes between inhaled fluid and blood in the pulmonary capillaries (138,162,163).

Chloride test, for example, was thought to be the most specific method by showing the difference of more than 25% of chloride concentration between the blood from both left and right chambers of the heart, expected to be positive in sea water drowning and negative in fresh water drowning because of haemoconcentration and haemodilution respectively (59). Although the method met some support, unfortunately the theory was not borne out in practice due to many facts which invalidate its

reliability. One of these was ascribed to the difference in the chloride content of various tissues. The shift in the direction of chemical homogeneity (143) is responsible for the phenomenon of progressive fall of chloride concentration as the interval between death and sampling lengthens (114). The difference of chloride concentration between left and right chambers of the hearts is likely to be masked by post-mortem diffusion (114), therefore unless chloride determination is made very early, the final judgment regarding its value is suspect (184).

It is suggested that disproportionate depression of chloride in the left side of the heart of 17 meq./l. or more is presumptive evidence of fresh water drowning (114). However, comparative studies on the left and right sides of the heart of drowning cases by using the chloride method, showed many of discrepancies in diagnosis of both fresh and salt water drowning (47), which might have resulted from partial settling of blood cells in the heart during post mortem interval. Also, inconsistent results had been obtained from cases recovered from different types of drowning media (47). Furthermore, artefact interference in determination of the chloride content might be anticipated in cases of aspiration of chloride-rich gastric content or inhalation of chlorinated water (112). The method is also useless when the water is brackish having a saline content of

about that of blood (59) , as any exchange between the alveoli and blood must be very limited (61). Therefore the determination of haemoconcentration and haemodilution by using chloride method is not satisfactory.

Other electrolytes such as sodium and potassium were examined on the same principle of predicted difference in concentration in both chambers of the heart. But, on critical review, many promising ideas related to the examination of these electrolytes have been discredited. Moreover, disproportionate intracardiac haemodilution of haemoglobin concentration and erythrocyte content of left and right sides of the heart were promised to be the best diagnostic method for drowning (17,127), but it has been discredited like other haemodilution methods.

Other workers suggested quantitative estimation of magnesium as being a more reliable test than chloride in salt-water drowning (114). However, the progressive increase of magnesium post-mortem is recognized after 6-12 hours after death. This is continued on putrefaction, but the rate of increase does not occur in the same manner in two sides of heart (114). This method, however, has been criticised on grounds of finding a slight difference in the magnesium concentration between the two sides of the heart in both fresh and salt water drowning.

In salt water, other researchers advocate plural fluid as a source for determination of chloride content, to avoid the controversial results usually obtained from blood (148). By using this method, a value more than 600

mg. of chloride per. 100 c.c. of fluid was being considered significant in cases of salt water drowning.

In other studies, instead of using blood from the right side of the heart to be compared with that of the left side, other biological fluids such as cerebrospinal fluid and vitreous humor were used with relative success.

1.8. PHYSICAL METHODS:

Application of physical methods such as examination of the specific gravity, freezing point and electrolyte conductivity for the diagnosis of drowning rely on the same idea of disproportionately diluted or concentrated blood of the left and right ventricles in fresh and salt water drowning respectively.

The specific gravity of blood in drowning is considered the simplest and most reliable test for diagnosis (47). Specific gravity of left atrial plasma in cases other than drowning tends to be higher than that of right atrial plasma, while in cases of drowning and regardless of salinity of water, it is always less than that of right atrial plasma (47). However, the test is not entirely satisfactory, because of many values that negate its value such as putrefaction which causes autolysis of plasma proteins, haemolysis with resultant haemoglobinaemia and the influx of glucose from the liver into blood. These complicate the picture and inconsistent results are readily predicted (111).

1.9. SURFACTANT:

The effects of inhalation of water in both types of drowning have been studied more thoroughly by studying the properties of "the lining complex" of the alveolar spaces.

The alveoli of the lung are lined with a film of high molecular weight lipoprotein the "lining complex" which has the function of lowering the surface tension. This complex is covered by a layer of surface-active material containing lecithin (128). This surface active material plays an important role in modifying the surface tension. Surfactant is a complex material. .But the principle surface active material of surfactant is thought to be dissaturated phosphatidylcholines (32). However it is not known whether this membrane has a selective permeable nature or not (143), though it plays an important role in the direction of movement of inhaled fluid and its solutes (128). Since the pulmonary surfactant is being washed out in salt water drowning, or the surface tension character is being altered in fresh water drowning, the effect will be for smaller alveoli to contract and larger ones to expand (110). Also, as a result of inactivation or dilution of surfactant due to fresh and salt water respectively, the compliance of the lung will decrease (114,163). An other effect of decreasing surfactant is through increasing intrapulmonary shunting which may be caused by atalectasis. These two effects i.e. decreasing

compliance and increasing intrapulmonary shunting (114,161,163) have explained to some extent the hypoxia as being the common lethal denominator in most cases of drowning.

The effect of inhaled water on surfactant has been examined by administration of distilled water intratracheally. This showed inactivation of surfactant while salt water and isotonic saline washed it out (60). An other possible effect of surfactant in drowning cases is pulmonary oedema which is thought to be caused by chemical and physical changes of surfactant, as well as by circulatory over-load caused by inhalation of water (60). It is known that the absence of a special means of lowering the surface tension would lead to collapse and transduation of fluid into alveoli (128). Also, as a result of inhalation of water , surfactant would exert an effect of air way obstruction by producing a foam-like admixture within the tracheo-bronchial tree.

This froth has several peculiar properties due to a film lining the bubbles which can exert a surface tension pressure sufficient to reduce the net surface tension nearly to zero. These bubbles had being obtained by various ways such as water being forced down the trachea into excised lung, lung perfused with saline and ventilated, phosgene, intravenous adrenalin and bilateral cervical vagotomy (32,60,128).

2. MATERIALS AND METHODS:

Necropsy was carried out to establish the diagnosis of drowning in 93 cases recovered from water (Tab.1). These same cases, also, have been examined for haemorrhage in the temporal bone which has been discussed in chapter 3. While for the diatom test, only 63 cases have been examined; the results will be discussed in the next chapter.

Necropsy was done on 63 bodies recovered from water, with revision of the autopsy protocols of 30 other cases, i.e. the diagnosis of drowning in the 93 cases was being assessed according to the post-mortem findings.

Most of the cases were recovered from the River Clyde, the main waterway in the Glasgow area. The study of these cases included the past history of the victims, circumstantial evidence, and necropsy findings. The information obtained from the police and eye-witnesses and the findings of post-mortem necropsy were essential to establish the diagnosais of drowning. In order to achieve this aim a co-operative team was established, including forensic pathologist, forensic toxicologist and the police.

3. THE RESULTS:

3.1. GENERAL EXAMINATION:

3.1.1. EXTERNAL EXAMINATION:

External examination of the 93 bodies showed froth exuding from the mouth and nose in 9 cases. This frothy

TABLE-1. SUMMARY OF CASES RECOVERED FROM WATER.

Case No.	Sex	Age	Type of Death	Month	Location of Drowning
DROWNING CASES					
1	M	12	A	JAN	Burn
2	F	54	S	JAN	Loch
3	M	52	?	NOV	River Clyde
4	M	17	A	JAN	R.C.
5	M	7	A	JAN	Swimm.Pool
6	M	51	S	FEB	R.C.
7	F	64	A	FEB	R.C.
8	M	60	A	MAR	R.C.
9	F	68	S	MAR	Bathtub
10	M	39	A	APR	Slush
11	M	27	A	MAR	Kelvin R.
12	M	4	A	APR	Burn
13	F	48	S	APR	Bathtub
14	F	60	S	APR	Bathtub
15	M	33	A	APR	Burn
16	M	19	S	MAY	Bathtub
17	M	31	A	MAY	R.C.
18	M	10	A	JUN	Canal
19	M	52	A	JUN	R.C.
20	M	60	A	JUN	R.C.
21	F	13	A	JUN	R.C.
22	M	51	S	JUN	R.C.
23	M	53	S	JUN	R.C.
24	M	40	A	JUN	Forth&C.
25	M	30	A	JUN	R.C.
26	M	63	A	JUL	Pond
27	M	45	A	AUG	R.C.
28	F	45	S	AUG	R.C.
29	F	75	S	SEP	Bathtub
30	M	35-45	?	SEP	R.C.
31	F	77	S	OCT	Loch
32	M	22	A	OCT	Sea
33	M	34	A	OCT	Sea
34	M	22	A	OCT	Sea
35	M	66	S	NOV	Loch
36	F	61	?	NOV	R.C.
37	M	27	A	OCT	Sea

38	M	73	S	NOV	R.C.
39	F	71	S	DEC	Forth&C.
40	F	37	S	DEC	R.C.
41	M	70	A	MAR	Forth&C.
42	M	14	S	APR	R.C.
43	M	44	A	MAY	Bathtub
44	M	17	A	MAY	R.C.
45	M	53	A	JUN	Fort&C.
46	M	23	A	FEB	R.C.
47	F	42	S	MAY	R.C.
48	M	22	A	JUN	R.C.
49	M	28	S	JUN	R.C.
50	F	73	S	AUG	R.C.
51	F	56	A	AUG	R.C.
52	M	18	A	AUG	R.C.
53	M	52	S	SEP	R.C.
54	M	74	A	SEP	River
55	M	14	?	MAR	R.C.
56	M	?	A	FEB	R.C.
57	M	49	A	JUN	R.C.
58	M	5	A	JUL	River
59	F	40	A	JUL	River
60	M	61	A	NOV	Fort&C.
61	M	67	A	MAR	R.C.
62	M	52	A	AUG	R.C.
63	M	39	A	SEP	River
64	M	23	A	DEC	Sea
65	M	58	A	MAR	R.C.
66	M	25	A	MAR	River
67	M	24	A	JUL	R.C.
68	M	18	A	OCT	Fort&C.
69	M	42	A	JUN	R.C.
70	M	8	A	AUG	R.C.
71	M	35	A	DEC	River
72	M	62	?	DEC	River
73	M	51	A	APR	R.C.
74	F	64	?	JUL	R.C.
75	F	75	A	JUL	R.C.
76	M	5	A	AUG	Fort&C.
77	M	77	S	SEP	Bathtub
78	F	28	A	SEP	R.C.
79	F	68	A	OCT	R.C.
80	F	31	A	NOV	R.C.

81	M	5	A	JAN	Kelvin R.
82	M	59	S	APR	Burn
83	M	26	A	MAY	Forth&C.
84	M	58	A	JUN	R.C.
85	M	66	A	JUN	R.C.
IMMERSION CASES					
1A	F	59	A	MAR	R.C.
2A	M	58	A	SEP	Forth&C.
3A	M	40	S	APR	Forth&C.
4A	M	7	A	DEC	Swimm.Pool
OTHER CASES					
5A	M	40	A	JUN	River
6A	M	74	A	JUN	Lake
7A	M	60	A	MAR	Ditch
8A	M	26	A	APR	Swimm.Pool

fluid was fine, clear and occasionally tinged with blood. Sometimes it could be demonstrated more clearly by pressing on the chest.

In 3 cases, the skin of the feet and hands was wrinkled, soggy and white. This phenomenon is called the "washer-woman's hand".

However it was absent in 63 bodies, though most of them had been immersed for a considerable period of time.

In 2 other cases the "degloving" phenomenon was shown where the skin of the hands was easily removed in one piece like a glove.

3.1.2. INTERNAL EXAMINATION:

Postmortem examination showed bulky and distended lungs in 65 bodies. These lungs were usually voluminous, water logged and ballooned to a degree that they overlaped the pericardium with indentations of the ribs impressed upon their surfaces. When removed and placed on the table, the lungs did not collapse.

They were usually pale with a spongy or dough-like feel. The pleural surface of the lungs in most of these cases showed a mosaic pattern of gray areas among red colored areas, caused by air trapping and the collapse of the alveoli respectively.

The cut surface of the paranchyma showed marked degree of congestion with oedema.

Typical hyperinflated lung was not seen in 28 bodies,

in which 7 were moderately to severely decomposed. In other cases, obliteration of the plural cavity by dense adhesions was the reason for absence of hyperinflation. In the remaining 17 cases, neither autolytic changes nor pleural adhesions were found to explain the absence of this sign in other negative cases.

An other finding in the air passages, the trachea, bronchi and bronchioles of 56 bodies was fine, clear and occasionally blood tinged frothy fluid. Also, fine to coarse frothy fluid was found in the air passages of 10 decomposed bodies. This type of frothy fluid did not differ from that which had been seen in freshly recovered bodies.

Examination of the pleural cavity showed a mild to moderate amount of blood stained fluid in 25 cases. This sign was not restricted only to those which showed hyperinflated lungs, as the latter was absent in 13 of those cases. Among those 25 cases there were 7 bodies with autolytic changes.

Examination of the stomach required special attention. The contents of it should be kept intact by tying its orifices before its removal from the rest of other viscera. The stomach had shown a moderate quantity of watery fluid in 49 cases. It, usually contained weeds and vegetation identical to those seen on the surface of the particular body. Also, recently ingested food and in large amount was found in the stomach of 5 other cases.

3.1.3. TOXICOLOGICAL EXAMINATION:

Toxicological examination of blood and other biological fluids was being done routinely in all cases recovered from the water.

Alcohol and/ or other drugs were detected in 42 cases, while drugs alone were discovered in blood or liver tissue in 8 cases. The types and levels of each drug is shown in table-2.

The discovered amount of drug varied from a therapeutic to a toxic level. In 6 cases, in which death was accidental, the level of drug was more or less within a therapeutic level. Only one suicidal case showed a toxic level of Temazepam.

Alcohol with other drugs was discovered in 4 other cases, in which a suicidal type of death was the case in 3 of them. As the fourth case had been recovered in a putrefied condition without enough circumstantial evidence, no cause could be found to support a particular type of death. The alcohol level in one of these cases was high in the blood and relatively low in urine, while in another case it was low in the blood compared to the level in urine. Two cases were found to contain only one drug as well as alcohol. Clomipramine in therapeutic level was found in one case, and dihydrocodeine in amount of 0.1mg was detected in the other case. In the 2 other cases, 2 or 3 drugs were detected together with alcohol, when 60 mg. paracetamol/100 ml. blood, 0.2 mg. of diazepam/ 100 ml blood and 333 mg. alcohol/ 100 ml blood

TABLE-2

SUMMARY OF LEVELS OF ALCOHOL AND OTHER DRUGS IN CASES OF DROWNING ,REGARDING MODE OF DEATH IN THESE CASES.

CASE	DRUG	LEVEL	TYPE OF DEATH	LOCATION
DROWNING CASES				
3	ALCOHOL CLOMIPRAMINE	25mg/100ml B THERAPEUTIC	(?)	CLYDE
6	ALCOHOL	426 mg/100ml B. 423 mg/100ml U.	S	CLYDE
9	TERPINEOL	?	S	BATHTUB
10	LORAZEPAM	1 µ/100ml.B	A	SLUSH
13	ALCOHOL DIHYDROCODINE	19 mg/100ml.B 147 mg/100ml U. 0.1 mg in liver	S	BATHTUB
14	TEMAZEPAM	HIGH LEVEL	S	BATHTUB
15	ALCOHOL	HIGH LEVEL	A	BURN
16	ALCOHOL	162 mg/100ml B.	S	BATHTUB
18	BUTANE	?	A	CLYDE
20	ALCOHOL	22 mg/100 ml.B. 11 mg/100 ml.U.	A	CLYDE
22	ALCOHOL	304 mg/100ml. B. 395 mg/100ml.U.	S	CLYDE
24	ALCOHOL	187 mg/100ml.B. 316 mg/100ml.U.	A	FORTH&CLY
25	ALCOHOL	284 mg/100ml.B.	A	CLYDE

27	ALCOHOL	28 mg/100ml 10 mg/100ml.U	A	CLYDE
28	ALCOHOL PARACETAMOL DIAZEPAM	333mg/100ml.B. 88 mg/100ml.U. 60 mg/100ml.B. 0.2 mg/100ml.B.	S	CLYDE
29	ALCOHOL	20 mg/100ml.B. 7 mg/100ml. U.	S	BATHTUB
34	ALCOHOL	HIGH LEVEL	A	SEA
37	ALCOHOL	33 mg/100ml.B	A	SEA
41	ALCOHOL	274 mg/100ml.B. 255 mg/100ml.U.	A	FORHT&CLY
42	ALCOHOL	44 mg/100ml.B 24mg/100ml.U.	S	CLYDE
46	ALCOHOL	101 mg/100 ml.B. 90 mg/100ml.U.	A	ClYDE
49	ALCOHOL	207 mg/100ml.B. 309 mg/100ml.U.	S	CLYDE
51	ALCOHOL	31 mg/100ml.B.	S	CLYDE
52	ALCOHOL	149 mg/100ml.B. 192 mg/100ml.B.	A	CLYDE
54	ALCOHOL	24 mg/100ml. B.	S	PIER
59	ALCOHOL	320 mg/100ml.B. 400 mg/100ml U.	A	River
61	ALCOHOL	230 mg/100ml. B. 298 mg/100ml.U.	A	RIVER
63	ALCOHOL	260 mg/100ml 463 MG/100ML	A	RIVER

64	ALCOHOL	19 mg/100ml.B.	A	SEA
66	ALCOHOL	38 mg/100ml. B.	A	RIVER
67	ALCOHOL	66 mg/100ml. B.	A	CLYDE
72	ALCOHOL	267 mg/100ml.B.	A	Quarry
79	ALCOHOL	300 mg/100ml.B.	A	CLYDE
83	ALCOHOL	307 mg/100ml. B.	A	FORTH&CLY
84	ALCOHOL	220 mg/100ml.B.	A	CLYDE

TABLE 30

IMMERSION CASES

1A	THIORIDAZINE	0.35 mg. liver	A	Clyde
2A	ALCOHOL	239 mg/100ml.B. 315 mg/100ml.U.	A	FORTH&CLY
3A	ALCOHOL PARACETAMOL MIANSERIN DIAZEPAM	93 mg/100ml B. 89 mg/l. B 0.66mg/kg.liver Trace amount	S	FORTH&CLY

OTHER CASES

5A	CHLOROPROMAZINE	0.15 mg	A	CLYDE
6A	LORAZEPAM	0.13 mg/l. B.	A	POND
7A	ALCOHOL	260 mg/100ml. B. 384 mg/100ml B.	A	DITCH
8A	CLOMIPRAMINE	Therapeutic level	A	SWIMM.POOL

were found in one case, while the other case contained 89 mg of paracetamol per. litre of blood , 0.66 mg. of mianserin per. kg. of liver and a trace amount of diazepam in the liver together with 93 mg. of alcohol / 100 ml. of blood.

Alcohol alone was found in blood and urine samples from 26 cases. The level was variable from one case to another, though it was nearly similar in both types of samples.

Alcohol above 100 mg/ 100ml. blood was found in 21 cases, 17 of which had a level more than 200 mg./ 100 ml. blood. The highest level was discovered in a man who committed suicide by jumping into a river with levels of 426 mg. and 423 mg. per. 100 ml. in blood and urine respectively.

3.2. BATHTUB DROWNING:

Seven bodies were discovered to have drowned in the bathtub. They were 4 females and 3 males . The type of death in 6 of these was suicidal, according to the circumstantial evidence. Four of them were under the effect of alcohol and/ or other drugs before their death. Alcohol was found in a high level in the blood of one case and in the urine of an other case. Temazepam was found in one of these.

One suicidal case was found to contain a natural oil, terpineol. This substance is usually found in

disinfectant, a bottle of which was found near the body. In 2 other cases no alcohol nor drug could be found. One of these cases was a child with spastic paralysis of lower limbs in which the finding of gastric content inhalation solved some of the ambiguity. In one other case neither circumstantial evidence nor necropsy findings supported any particular type of death.

Postmortem examination of one of the cases, a 5 years old child with history of spastic paralysis, showed no signs of inhalation nor swallowing of water. The trachea and bronchi contained a considerable quantity of regurgitated gastric contents identical to that seen in the stomach. The lungs did not showe the ballooning characteristic of drowning, but on serial slicing small amounts of fluid and gastric contents were easily expressed from the smaller bronchi and the larger bronchioles. The stomach was fully distended by a recently ingested meal.

In other cases, postmortem examination showed hyperinflated and bulky lungs in 5 of them. The air passages in these cases contained a fine frothy fluid, sometimes in abundant amount. Moderate amounts of watery fluid was detected in the stomach of 4 bodies. There was only one case with a moderate degree of decomposition which showed blood stained froth exuding from the mouth and nose.

3.3. SWIMMING POOL DROWNING:

Three subjects were recovered from swimming pools in an unconscious condition, dying a few minutes later despite resuscitative measures.

The first case (No.5) was a 5 years old male child who was found lying at the bottom of the pool after he had been seen jumping into the water several times. Postmortem findings showed a moderate amount of blood stained fluid in the pleural cavities with a fine froth in the trachea and bronchi. The lungs were bulky and distended. No water was found in the stomach. However there was evidence of wide spread inhalation of gastric contents into the lungs. No other pathology was found which might have incapacitated him or caused him to collapse.

The second case, No. 8A, was a 28 years old male. He, also, was found lying on the bottom of the pool. Despite vigorous resuscitation, he died soon afterwards. Postmortem examination showed clear and dry pleural cavities. The trachea and bronchi contained an abundant amount of blood stained froth. The lungs were well expanded and showed also gross inhalational type haemorrhage with massive oedema. The gastric contents were also demonstrated in the air passages. The stomach contained a moderate amount of watery fluid. Other organs were unremarkable, apart from mild atheromatous changes of the coronary arteries.

Case No. 28 was a 4 year old child who was removed

unconscious from a swimming pool. Despite resuscitative measures he did not regain consciousness and died on arrival at the hospital.

At autopsy there was a considerable quantity of regurgitated gastric contents in the air passages. Both lungs were heavy and congested and no excess of water was found in the stomach.

4. DISCUSSION:

4.1. EXTERNAL:

On external examination we concentrated on "washerwoman" changes and froth exuding from the mouth and nose, as those being the most common features.

Other external signs, which have been mentioned in almost all the textbooks and literature of forensic medicine, are thought to be of less significance in the diagnosis of drowning. These include cold pale skin, pink colored hypostasis, retraction of penis, scrotum and papilla mammae (59,61,96,143).

Lividity in drowning, for example, was found to have no distinctive appearance from other causes of death. It develops in any submerged body regardless of cause of death. It occur even after refrigeration which is a well known cause of pink lividity. It might be due to the cooling of oxygenated blood in the capillaries of skin. However, it seems to have no diagnostic significance. Also, no significant value has been attached to a

phenomenon called "cutis ansernia" which is caused by contraction of the erector pilae muscles. Since the rigor of the erector pilae muscles can produce a similar reaction, it is not uncommon and is found in many other forms of death. It is the same mechanism that produces the appearance of the skin in cold conditions during life. It has no value in determining whether or not the person was alive at the time of immersion.

The other phenomenon of immersion was the wrinkled, soggy and white appearance of the hands and feet which was observed in 30 cases. This phenomenon is due to imbibition of water into the skin leading to the swelling of the outer keratin layer (84). It has no value whatsoever in determining whether the victim was alive at the time of immersion.

There is another sign called cadaveric spasm which is unrelated to immersion though it may occur in drowning. It is described as the sudden agonal contraction of the muscles of the arms and hands causing the deceased to clutch at objects whilst he is either falling into the water or drowning.

It could not be demonstrated in any one of 93 cases, but it was not uncommon to find vegetation entangled between the fingers. It is not considered as a vital phenomenon due to the fact that muscle relaxation at death with passive movement of the submerged body and objects like weeds remain entangled between the fingers rather than being clutched by the so called cadaveric

spasm.

It is confirmed that cadaveric spasm is a rather rare phenomenon and it cannot be relied on in cases of drowning.

4.2. INTERNAL:

4.2.1. THE FROTH:

A fine, clear and white foamy fluid exuded from the mouth and nose of the 9 bodies whose lungs were hyperinflated. It may be present at the time of recovery of the body, or if absent, appear later especially after compression of the chest.

Also, foamy fluid was observed in the trachea, bronchi and bronchioles of 56 bodies. Sometimes it could be expressed easily from the main bronchi after the lungs had been removed and placed on the table. This sign is thought to be characteristic of drowning (65,66,148). However it was difficult to distinguishe from the froth produced by the process of decomposition as it had been seen in 10 putrefied bodies. Furthermore the sign was absent in many cases of undoubted drowning.

Since the froth is in fact a sign of pulmonary oedema, it may be seen in a number of conditions. The froth consists of protein and water churned into a mass of bubbles by respiratory contraction aided by the presence of surfactant. It is considered as a vital reaction, in that respiration is a prerequisite for its formation. However the froth alone cannot be taken as prima facie

evidence that the victim was alive at the time of immersion. Even so, we still consider, (in agreement with others opinion) (23,132), the presence of froth is highly significant if the circumstances of death are such as to suggest drowning.

4.2.2. VOLUMINOUS LUNG:

The lungs of 66 bodies were bulky and voluminous and showed a mild to moderate degree of congestion and oedema. The so called mosaic appearance over the surface of the lungs was the usual feature of these voluminous lungs. This phenomenon is due to mottling of the collapsed or oedematous alveoli amongst those having been distended with air. The cut surface usually revealed marked congestion with frothy fluid. The overall picture of the lung has been described as emphysema aqousum, trokenes modem or emphysema hydroaerique. It was observed in nearly 70% of our cases which let us conclude that hyperinflated, voluminous, or drowning lung is nearly a characteristic sign of drowning. However the main point of controversy is between drowning lung and a similar condition called hydrostatic lung which may occur in submerged bodies which died from causes other than drowning (136,137). The term hydrostatic is derived from a flow of water into the air passages under the hydrostatic pressure of water. During drowning, what usually happen, is that the fluid penetrates deep into

the lung even to the complete occupation of all the alveoli, while in hydrostatic lung, only a trace amount of water gravitates to the dependent part of the lung (61).

Drowning lung , however, was absent in 27 cases, 9 of which were in a decomposed condition, i.e. the signs of drowning lung were negative in 18 freshly recovered bodies. On the other hand voluminous lungs were seen in 9 decomposed bodies in which the sign could not be totally ascertained whether it was drowning lung or hydrostatic lung. This differentiation is difficult in the presence of other signs like froth or water in the stomach, which might be produced postmortem. However, in these 18 cases, dry drowning was suggested. Also, there was a possibility that they had died as a result of immersion, but from causes other than drowning where no signs of inhalation or swallowing of water could be demonstrated. However we should permit that in most of cases, the diagnosis of drowning is nearly always made by exclusioneum.

4.2.3. WATER IN THE STOMACH:

A moderate amount of watery fluid was found in the stomach of nearly 51% of the cases. In most, the watery fluid contained vegetation similar to that found on the surface of the body. This sign may have some significance, if it supplies a characteristic for identification.

It may indicate a vital process after submersion, but

it was just as often absent, as present, in drowning victims. This sign has no significant value in the diagnosis of drowning. Its value is also limited by the finding that the fluid may enter stomach after death (64).

4.2.4. CARDIAC DILATATION:

Cardiac dilation as a result of fluid aspiration obstructing the pulmonary circulation and causing acute right ventricular strain appears to be mediated by both an anatomical and vasomotor increase in the pulmonary vascular resistance. The anatomical component stems from the wide spread obstruction of small pulmonary arteries and arterioles, whereas the vasomotor increase in resistance is the result of severe hypoxia attendant upon fluid aspiration which act as a potent stimulus to pulmonary vasoconstriction (44).

True cardiac dilatation can be recognized at necropsy only when it occurs in association with certain forms of cardiac hypertrophy. The distension of auricles and ventricles is a common post-mortem phenomenon which might result from secondary muscle flaccidity. Therefore, even if met with in cases of drowning, it would hold little significance. It is in general, an unreliable sign.

4.2.5. FLUIDITY OF BLOOD:

The fluidity of the blood at necropsy depends on the concentration of fibrinolysis and the rate of intra-

vascular coagulation after death (67). It is thought that production of fibrinolysin might be part of a nonspecific general reaction to injury which characterizes Sely's alarm reaction (67). This reaction is defined as the sum of all nonspecific system phenomenon following sudden exposure to stimuli to which the organism is quantitatively or qualitatively not adapted. Therefore postmortem fluidity of blood is not characteristic of any special cause or mechanism of death. In drowning it might be due to absorption of water into the blood during the agony. Regarding the exact mechanism, it was concluded from a series of studies on cadaveric blood that the fluidity of blood is due to fibrinolysis activated by the release of plasminogen activators. Such activation occurs as a specific reaction mediated by various vasoactive materials (167) which act on receptors situated in the vascular walls. One of these vasoactive materials is a catecholamine which is produced in large quantities during the agonal period especially in cases of violent death.

Fibrinolysis activation is also produced by the leakage of the plasminogen activator due to enhanced permeability, degeneration or necrosis of the cell membranes as a result of excessive acidosis after death (167). This acidosis is a constant finding in cases of drowning. Acidosis of the blood is also commonly found in nearly all types of death. For that reason there is no

specific mechanism related to drowning. It is therefore not a reliable sign and has little significance in drowning.

4.2.6. PETECHIAL HAEMORRHAGE:

Petechial haemorrhages in the visceral pleura and pericardium have been regarded as pathognomonic signs of death from mechanical interference with respiration. These petechiae are called Tardieu's spots. These spots have been recorded in other causes of death. However it is held that the ones observed in cases of mechanical interference with respiration differ in number, size, colour and distribution.

In this study, petechial haemorrhages could not be demonstrated in any case recovered from the water. These results confirm the view that these spots are always infrequent, and indeed rare. The explanation for this scarcity may be due to compression of the interalveolar septa, or because the convulsive phase is brief and less violent in drowning than in other asphyxial deaths.

Since these petechiae are considered to be rare in drowning, they hold little value as a characteristic sign of mechanical interference with respiration by aspiration of water. Furthermore, even if they are present at necropsy they cannot be considered as an antemortem phenomena as they commonly develop after death and even during dissection (67).

Another type of haemorrhagic spots which are mentioned

to occur in cases of drowning (173), are called Palatuf spots. These are large, shining and pale bluish-red. They may be minute or of 3-5 cm diameter and are usually present in the lower lobes of the lungs. However they were absent in all the present series cases recovered from the water which confirms their scarcity in drowning and affirm their unreliability for diagnosis.

4.3. SWIMMING POOL DROWNING:

The swimming pool is considered the most common locus of drowning in children under the age of 10 years (34,35). In the current study it was reported in only 3 out of 93 cases. There are, however, some interesting findings in these cases which are worthy of discussion.

The first case was a child aged 5 years. Necropsy showed classical signs of aspiration of water, with evidence of widespread inhalation of stomach contents into the lungs. Given the reported circumstances, it would appear that this patient was still alive when removed from the water, but subsequently vomited and inhaled the vomit into his lungs. Before that, he had jumped into the water several times and was found shortly afterward lying at the bottom of the pool near the deep end. No cause could be found which could have incapacitated him or caused him to collapse. The stomach contained a moderate quantity of recently ingested food-stuff but no water. By virtue of finding regurgitated food in the air passages, it is not known whether this

happened as result of drowning or as result of resucistative measures.

The possibility of inhalation of gastric content as a cause of drowning has been also raised in the second case.The victim was 28 years old male who was found lying at the bottom of the pool. Despite vigorous resuscitation he died soon afterwards. Postmortem examination showed that he died ultimately as result of inhaling vomit into the lungs, this having produced massive pulmonary haemorrhage. Although it remains a possibility that for some reason he did in fact inhale water and partially drowned, and that this and/ or attempts to resuscitate him caused him to vomit and subsequently inhale the vomit. The cause of death was issued as pulmonary haemorrhage and oedema due to inhalation of stomach contents.

The third case was a child aged 7 years, who was removed unconscious from a swimming pool, but did not regain consciousness despite attempts of resuscitation, and died on arrival at hospital.The cause of death was reported as immersion in water and the inhalation of gastric content.There was, however , no evidence that he died directly from drowning.

We learn from these cases that the inhalation of gastric contents is common in swimmers after ingestion of a fairly large meal. However it is still uncertain whether the vomiting was initiated as a result of

drowning, or by attempts of resuscitation which had been conducted on all these three cases in which inhalation of water could not be demonstrated in the air passages. Also, there is a slight possibility of the so called hyperventilation death, which is highly probable in cases of swimming pool drowning. In such instances the person hypreventilates, before a breath-holding exercise. This may delay the onset of the urge to breathe and before the partial pressure of carbon dioxide increases significantly, and the oxygen decreases to a level incompatabile with cerebral functions. Awareness of the urge to breath is mainly due to the partial pressure of carbon dioxide in the blood with comparatively little stimuli being derived from oxygen (61).

4.4. BATHTUB DROWNING:

The classical signs of inhalation of water was demonstrated in the 5 cases found dead in a bathtub. The signs included bulky and distended lungs, froth in the air passages and a moderate amount of water in the stomach. In one other case, despite decomposition changes, there was no evidence of injury on the body and no sign of other disease. He would appeared to have died from drowning as the only possible cause of death, based on the exclusion of any other causes.

The seventh case was a child aged 5 years who had a history of spastic paralysis of the lower limbs. He was found submerged in a bathtub . On examination the trachea

and main bronchi contained a considerable quantity of regurgitated gastric contents identical to that seen in the stomach. The lungs did not show the ballooning, characteristic of drowning.The cause of death, therefore, was partly a result of drowning and partly from regurgitation of gastric contents.

Death in a bathtub should be analysed carefully as it is essential to differentiate between the different types of drowning by determining the causes leading up to it. This view has been emphasized by other workers as well (42). Many causes of death in a bathtub have to excluded before deciding that drowning is the only cause. These included carbon monoxide poisoning, electrocution, alcohol intoxication, abortion, other natural diseases and homicide by drowning.

It seems that females are more involved with this type of drowning. Alcohol and/ or other drugs were found in 4 cases of suicidal bathtub drowning. It is rarely for someone to drown him or herself without preceding conscious-weakening measures unless he or she is strongly motivated. This variety of drowning was determined in one case in which neither postmortem findings nor circumstantial evidence supported any other type of death. In bathtub drowning, attention should be paid to the precise cause of death, whether it is accidental, suicidal or criminal. It is recommended that examination by a forensic pathologist and toxicologist should be carried out promptly in these cases, as a bathtub is a

desirable place to conceal murder. Also the possibility of feigning accidental or natural death by placing an unconscious person in a bathtub is sometimes difficult to exclude.

The diagnosis is more difficult in victims who die from circulatory collapse, syncopeal attack or shock. The latter is termed classical bathtub murder (69); it is diagnosed usually by exclusion.

4.5. ALCOHOL AND OTHER DRUGS:

The positive results of alcohol, obtained from 26 bodies, confirm the view that alcohol is the most common factor associated with drowning (170,186). The incidence of alcohol- related drowning is more or less the same in countries like Norway, Finland,England and scotland with percentages of 20%, 30%, 29% and 21% respectively (170). The World health organisation (WHO) reported that as many as 15,000 victims out of 140,000 drowning deaths per.year are alcohol related (170). For example in Scotland, in 1983 there were 91 alcohol-related cases in 485 drowning cases (170). However, the problem may be bigger than these figures as the association between drinking and drowning is often underestimated , since blood alcohol concentration is not always estimated and a reliable history may not always available.

Though the lower limit of the lethal concentration of alcohol is 350-400 mg./100 ml. of blood, more or even

less than this was found to predispose to death by drowning. It is known that alcohol has a depressant effect on the central nervous system which may lead to paralysis of the higher centres if its level in the blood reaches a toxic concentration. As a low level alcohol has a depressant effect on gluconeogenesis which causes a fall in blood glucose and leads to weakness and confusion which might therefore cause drowning. The effect of alcohol on thermoregulation has been shown to be a further complication (90,91).

Instantaneous death or badetod has been described in alcohol-related immersion, in which the victim dies suddenly without panic and without aspiration of water. Notwithstanding classical signs of inhalation of water were observed in most cases of alcohol-related drowning.

One of possible explanations of alcohol related drowning is the vasodilatory effect on the skin with a rise in the temperature which may produce abnormally strong stimuli acting on the cold receptors of the skin. These stimuli may lead to uncontrollable hyperventilation and subsequent inhalation of water. They may also cause increase in venous and systemic blood pressure, increase in pulse pressure and ventricular fibrillation. This may in turn cause inhalation of water or collapse.

4.6. IMMERSION IN COLD WATER:

Immersion in cold water and hypothermia being are problems associated with drowning

(61,63,90,91,92,109,146). Most attention has been paid to the effect of cold water immersion after the tragic sequel to the Titanic disaster in 1912.

It was thought that several mechanisms were involved in sudden death on immersion, such as cold anaphylaxis and other reflexes which might cause cardiac arrest. This mechanism of death is not accepted by others who believe that ventricular fibrillation is the real cause of death (90,91,92).

Other factors such as myocardial infarction, cerebrovascular accident or tetany have been suggested as contributory factors leading to drowning in cold water immersion (63). It is established that sudden immersion in cold water cause tachycardia, ectopic heart beats, increase in arterial blood pressure and cardiac output (91,92). Other physiological changes,such as intense hyperventilation, tachycardia, peripheral vasoconstriction and subsequent increase in cardiac output have been observed on cold water shower bathers (90). These cardiovascular changes are confirmed to be caused by reflexes (92). These reflexes are initiated by cold receptors in the skin which are more numerous in the chest and abdomen. These receptors are responsible for the initiation of such stimuli which are mediated through the tegmentum of midbrain and the hypothalamus (92). Similar changes also had been obtained experimentally. These reflexes in turn lead to stimulation of sympathetic nerves which increases the

force and frequency of cardiac contraction (92). The hyperventilation, with an inspiratory shift in respiration and dyspnoea are potent reflexes, which can lead to inhalation of water on sudden and complete immersion in cold water.

4.7. OTHER PATHOLOGICAL CHANGES:

Different pathological changes have been discovered in most bodies recovered from water; usually of the coronary arteries and heart muscle. They were in the form of atherosclerotic changes of variable severity. Recent thrombosis was discovered in the left coronary artery in 1 case. Old myocardial infarction with moderate atheromatous changes of coronary arteries was found in 5 other cases. Fracture of the skull with laceration of the brain was observed in 2 cases. Blotchy purple patches on the forearms and knees attributed to hypothermia were seen in 2 cases . Haemorrhagic changes in the lung were found in 2 instances while regurgitation of food content into the air passages was detected in 4, including the cases with haemorrhagic lungs. One case presented with retroperitoneal haemorrhage due to fracture of the spine.

Necropsy revealed in 8 of these cases only a few or none of the classical signs of the inhalation of water. Other internal and external morbid changes suggested causes of death other than drowning.

These cases are described as follow :

1. Case No. 1A was a 59 years old female who had a long history of psychiatric illness and in the past had attempted suicide . Her body was found floating in the River Clyde. Postmortem examination revealed few classical changes of drowning, the only indication being some fluid in the lungs and stomach. She also had evidence of severe ischaemic heart disease with extensive scaring of the myocardium. At the time of her death she was possibly slightly intoxicated by thioridazine which may have made her drowsy and confused. It is likely that this played a major part in her death; and given the circumstances, she probably died almost immediately after entering the water. It is just possible that she collapsed from heart disease and fell into the water. Although the clear suicidal history indicated otherwise, the death was certified as immersion in water and ischaemic heart disease due to coronary artery atheroma.

2. Case No. 2A was a male aged 58 years. He had a long history of chronic bronchitis and excessive drinking. He was found dead floating in the Forth and Clyde canal half a mile from his home. Postmortem examination showed few signs of drowning which were not prominent. This suggested that he died very soon after entering the water, a possible reason for this being that he was suffering from severe chronic lung disease such that it would have needed only small aspiration of water to cause severe respiratory distress. He was also considerably

intoxicated by alcohol which contributed to his death. The cause of death was certified as immersion in water, chronic bronchitis and emphysema and acute alcohol intoxication.

3. Case No. 3A was a male aged 40 years, who had a history of depression. Four weeks before his body was found in a canal, he was seen by someone rushing toward the canal and then disappearing. Postmortem examination showed none of the classical features of drowning. However as at that time of year the water was very cold, his death was most probably immediate on entering the water due to stimulation of the vagus nerve i.e. "vaso-vagal shock". The cause of death was issued as immersion in water.

4. Case No. 4A was a 7 years old male child. He was removed unconscious from a local swimming pool and later died on arrival at hospital. There were no signs of inhalation nor swallowing of water. As there was no evidence that he died directly from drowning, the diagnosis was made of immersion in water and inhalation of gastric contents, in virtue of finding the latter in the air passages.

5. Case No. 5A was a 40 years old male. He had a long history of schizophrenia, and on previous occasions he had threatened suicide . One day he was found lying on the rocks at the bottom of a water-filled quarry. Postmortem examination showed that death resulted from severe head injuries with fracture of the skull and

damage to the underlying brain. This may have rendered him unconscious and subsequently he inhaled blood coming from the fracture at the base of the skull. No other signs which suggested death by drowning could be found. Therefore the cause of death was certified as inhalation of blood with multiple injuries due to a fall from a hight.

6. Case No. 6A was a 74 years old male. He had a history of depression for which he had been prescribed lorazepam tablets. One day he was found lying semiconscious on a grass bank, partly dressed, with the reminder of his clothing on the ground nearby. He was taken to hospital but was dead on arrival. Postmortem examination showed that he had substantial ischaemic heart disease with evidence of old myocardial infarction and occlusion of one coronary artery. No convincing signs of inhalation of water was found apart from a moderate amount of watery fluid and a stone which measured (2.5 cm.x 2.5 cm.x 3 cm.) detected in the stomach. There was however a high level of the drug lorazepam in the blood consistent with him having taken an overdose of these tablets, sufficient to cause death. He also showed signs of hypothermia e.g. blotchy purple patches on the forearms and knees.The most likely explanation for his death was that he took an over dose of lorazepam tablets, and while unconscious developed a degree of hypothermia. It is difficult to explain the partial undressing other

than to suggest that his first intention may have been to commit suicide by drowning. The death was recorded as lorazepam intoxication, accidental hypothermia and ischaemic heart disease.

7. Case No.7A, was a 60 years old man. He was found dead, lying face down in a water filled-ditch. Examination of the body showed some changes in the skin suggestive of hypothermia, similar to those found in case No. 65. There was also, some, though not classical evidence of inhalation of water. The lungs were bulky and distended with sharp margins and showed mild venous congestion and severe oedema. He also had an enlarged heart and at the time of his death was considerably intoxicated by alcohol. There may have been a number of factors involved in his death, but the sequence of events was probably that, while intoxicated by alcohol he had fallen to the ground and lain face down in the ditch where he died sometime later; a combination of hypothermia and drowning.The fact that he also had heart disease reduced his chance of survival. Accordingly the cause of death was issued as immersion in water with accidental hypothermia, acute alcohol intoxication and hypertensive heart disease.

8. Case No. 8A was a 28 years old male who was seen lying at the bottom of a swimming pool. The case has been described previously with other swimming pool cases.

The important point here was related to the cause of death which was certified as pulmonary haemorrhage,

oedema and inhalation of stomach contents. The diagnosis was based on the finding of gross inhalational type haemorrhages due to aspiration of vomitus. Though the necropsy showed abundant blood stained froth in the trachea and bronchi, the lungs were well expanded with massive oedema, his death was not considered primarily due to drowning, although it remains a possibility that for some reason he did in fact inhale water and partially drowned.

According to the findings of these patients who died from causes other than drowning, attempts have been made to broaden the classical concept of drowning by incorporating into description of the post-mortem findings speculative theories about mechanisms of death. Also attempts have been made to distinguish between classical drowning and what is called atypical drowning. However drowning as a cause of death is in fact a result of an interplay of antecedent factors. Therefore there are questions requiring answers; 1.why the victim get into trouble in the first place ? 2.where in trouble, why could not the victim extricate himself or herself ?. Accordingly, the investigation scheme is an equation in which the result, drowning, is a constant and human factors plus environmental factors are the variables. The consideration of human variables, for example, will give many alternatives so far as the real cause of death is concerned. The interpretative approach to these variables, taking heart diseases as a common example, is

to gain as much information from as many sources as possible and then judge the alternatives. Therefore the resultant death may be listed as drowning by itself, drowning with contributory effect of cardiac disease, cardiac disease with contributory effect of drowning, cardiac disease with the contributory effect of aspiration of water and cardiac disease. Sometimes cardiac- induced drowning is too subtle to be readily disclosed during the necropsy. The heart may appear grossly normal and thus mislead the pathologist into disregarding it as potential human factor.

Regarding the diagnosis of drowning all signs of submersion have no evidential value on determining that the victim was breathing at the time of his submersion. In our results, the anatomical changes have a limited usefulness in determining presumptive evidence of drowning.

We admit that our concern is not primarily to know whether the person died from ventricular fibrillation or from myocardial anoxia, a difference which could not possibly be manifest naked-eye. Our concern, in fact, is to establish convincing vital signs of inhalation of water as being the primary cause of drowning. These signs, however, tend to be neither pathognomonic nor frequent which creates a real diagnostic problem. The picture is sometimes made more complicated by the finding of other pathological changes which may lead subsequently

to speculative theories about the different mechanisms of death which may occur when a body is submerged or immersed in a fluid medium

5. CONCLUSION:

Ninety three bodies recovered from water were examined to establish the cause of death after all the anatomic, toxicological and circumstantial evidences had been carefully assimilated and assessed.

The following results were obtained;

1. Eighty five cases were diagnosed as death by drowning. This diagnosis was based partly on the demonstration of the classical signs of inhalation or swallowing of water and partly on the exclusion of other causes of death.

2. Four cases had been diagnosed as immersion in water, referring to the condition of sudden death which is sometimes called hydrocution or vasovagal inhibition. The diagnosis is regarded as a primary cause of death with or without other pathological changes having contributed materially.

3. Four cases were identified with causes of death other than drowning or immersion in water. The diagnosis was based entirely on the finding of natural or unnatural causes, sufficient to cause death on their own.

4. All the bodies , who were found in a bathtub, were diagnosed as drowning cases in accordance with the postmortem findings and exclusion of other causes.

Whilst in the three bodies who were recovered from a swimming pool the diagnosis of drowning was justified in only one case. Immersion in water was the cause of death in one other case while pulmonary haemorrhage due to inhalation of gastric contents was the cause in the third case.

5. Postmortem examination in 18 bodies who showed moderate to severe degree of decomposition was difficult and sometimes futile, as they failed to demonstrate the classical signs of drowning.

6. Concerning the mode of death in cases recovered from the water, we found that drowning or immersion was accidental in 62 cases (64.5%) and suicidal in 24 cases (25.7%). In the other 7 cases neither circumstantial nor anatomical or toxicological investigation were able to identify the mode of death.

7. By toxicological analysis, alcohol or other drugs were detected in 42 cases, amongst these, 13 had drowned in a suicidal pact.

8. External examination in these cases revealed "washerwoman" changes of the hands and feet in 31 bodies (33.3%). This sign seemed unrelated to the time of submersion.

Froth exuding from the mouth and nostrils, a classical external sign, was seen in only nine cases, i.e. (9.6%).

9. Internal examination showed voluminous and hyperinflated lung in 65 cases (67.7%). These changes are due to inhalation of water, pulmonary oedema and air

trapping.

Fine, white and sometime blood stained froth was observed in the trachea, bronchi and bronchioles of 55 cases (59.1%). A moderate amount of blood-stained fluid was found in the pleural cavity of 26 bodies (27.9%).

In 49 cases (52.6%), the stomach was occupied by a moderate amount of watery fluid.

These external and internal signs were hardly classical nor frequent. They are regarded as supportive evidence of death by drowning, although the diagnosis is usually confirmed by exclusion of other causes of death.

These signs also cannot be used to determine accurately if a person was breathing at the time of his immersion in a fluid medium. Anatomical signs, in general, have a limited value in determining the presumptive evidence of drowning.

SECTION 1

HISTOLOGICAL EXAMINATION OF THE LUNG IN DROWNING AND OTHER ASPHYXIAL CASES

1. INTRODUCTION:

Deluge studies have been carried out to explore the morbid changes in the pulmonary alveolo-capillary epithelium following the aspiration of water, in the hope of confirming a diagnosis of drowning (84). The aim was also to differentiate between fresh and salt water drowning.

Experimentally, microscopic examination in fresh- water drowning revealed cellular disruption, mitochondrial swelling and endothelial destruction, attributed to the effect of osmosis. In salt water drowning the formation of vacuols and discontinuity of alveolar lining cells were the prominent features, although there is still preservation of the lung structure (84).

Some progress has been made by experimental examination of lung in fresh and salt water drowning by using the electron microscope, since morphological changes correlated well with the tonicity of the drowning medium.

In order to detect specific signs in drowned lung for classification as well as differentiation between typical and atypical drowning, the silver impregnation method of Gomori was proposed (137). The method was used to examine the reticular fibres thought to give different morbid

changes related to the degree of distension of the alveolar walls. This method, however, has not universal acceptance. Similar pathological changes have been seen in conditions other than drowning, e.g. aspiration of blood, capillary bronchitis, fatal asthmatic attack, protracted hypoxia from smothering, strangulation and chronic bronchostenotic emphysema (61).

The important findings in drowning are acute dilation of the alveoli with elongation and thinning of the septa and compression of the alveolar capillaries (84)

Other studies have revealed additional findings including swelling and intracytoplasmic vesicular formation, detachment of pneumocytes, swelling and vesicular formation of capillary epithelium in the alveolar septa, intralobular haemorrhages. perivascular and peribronchial oedema and general haemolysis (84).

Histoenzymatic study in experimental animals had promised to be a valuable diagnostic tool, not only in diagnosis, but also in differentiating between fresh and salt water drowning (153), but so far it has failed (like other methods) in accomplishing this aim. However, there are generally no constant and definite histological signs predictable in all cases of drowning because of the presence of capricious variables. These variables include the depth of the water, resuscitative measures, the general state of preservation of corpses, previous illnesses and earlier pathology.

2. MATERIALS AND METHODS:

This series include death by drowning,cardiac failure, brain injury, suffocation, strangulation, hanging, intoxication by alcohol, thyophylline, prothiaden, distalgeic and carbon monoxide, gas explosion, acute pulmonary oedema as the main pathology. It also covers miscellaneous causes, including diabetic ketoacidosis, peritonitis, pulmonary thromboembolism, acute hepatic failure, cot death and shock lung.

Necropsy was conducted in the normal way. Gross examination was made immediately to be followed by microscopic examination in order to determine if any previous or recent disease accounted for the oedema.

Blocks were taken from each lung lobe and fixed in 10% formalin for 24 hours. The tissues were then cut into thin rectangular blocks about 1/4 inch. thick, placed in small containers and again fixed in 10% formalin for a further 24 hours. After fixation the tissues were taken into a histokinette machine for automatic processing.

Sections were cut at 4μ and then stained with hematoxylin and eosin. Microscopical findings are recorded in the table-3 .

3. RESULTS:

The 118 cases were arranged under the headings of drowning, cardiac diseases, mechanical asphyxia, inhalation of gastric content, brain injury and

TABLE 3

SUMMARY OF PATHOLOGICAL FEATURES OF THE LUNG IN CASES OF SUDDEN ANOXIAC DEATH.

No.	AGE	SEX	OEDEMA	CONGEST.	HAEMORR.	ALV.PHAGOCY.
DROWNING						
1	68	F	+	–	–	–
2	39	M	+++	+	–	+
3	4	M	+	–	–	–
4	48	F	+	++	+	++
5	33	M	–	–	–	–
6	10	M	+	+	–	–
7	13	F	+	–	–	+
8	51	M	+	++	+	–
9	53	M	++	–	–	++
10	77	F	+	–	–	–
11	22	M	+++	++	–	–
12	34	M	+++	–	–	++
13	66	M	+	–	–	+
14	61	F	+	–	–	–
15	71	F	++	+	–	–
16	70	M	+	+++	–	+
17	40	M	++	++	–	++
18	44	M	+	++	+	++
19	60	M	+	++	–	++
20	32	M	+++	–	–	+++
21	45	F	++	–	–	+++
22	58	M	+	+	+	–
23	75	F	+	–	–	+++
24	35	M	++	–	–	+++
25	74	M	+	–	–	+
26	18	M	+	–	–	++
27	52	M	+++	+	–	–
28	17	M	+	+++	–	++
29	14	M	+	++	–	–
30	53	M	+	++	–	+++
31	26	M	+++	+	++	–
32	17	M	++	–	–	+++

NO.	AGE	SEX	OEDEMA	CONGEST.	HAEMORR.	ALV.PHAGOCY.
33	23	M	+	-	-	+
34	42	F	+	-	-	++
35	73	F				
36	36	M	+	-	-	-
37	55	M	+	++	-	+
38	19	M	+	-	-	-
39	49	M	+	++	-	+
40	40	F	-	++	++	++
41	52	M	+	-	-	+
42	18	M	+	+	-	-
43	42	M	+	+++	+	+++
44	8	M	++	-	++	+
45	35	M	+++	-	-	++
46	5	M	++	—	—	—
47	68	F	—	—	+	-
CASES OF HEART DISEASE						
1	31	M	+++	+++	-	-
2	53	F	++	++	-	++
3	58	F	++	++	-	-
4	72	F	++	++	-	+++
5	48	M	++	-	+++	++
6	31	M	+++	+++	-	-
7	45	M	++	++	-	++
8	64	F	+++	++	-	+
9	78	F	+++	++	+	+
10	31	M	++	+	-	-
11	54	M	+	+++	-	-
12	18	M	++	+++	+++	-
13	62	M	+	+++	+	+
14	73	M	+	++	-	+
15	60	M	+	++	+	-
16	72	M	++	++	+	-
17	57	M	+	++	-	+
18	39	M	++	-	-	+++

CASES OF ASPHYXIA (HANGING, STRANGULATION & SUFFOCATION).

1	20	M	+++	++	-	+++
2	9W	M	++	++	+	-
3	30	F	+	++	+++	+++
4	18	M	++	+	+	-
5	7	M	++	++	++	-
6	4	M	++	++	+	-
7	9W	M	++	+	+	+
8	18	M	+	+++	-	-
9	44	F	+++	+++	-	+++
10	74	F	+	++	+	-
11	45	M	++	++	-	+++
12	30	M	++	++	+	+
13	31	M	++	++	+++	++
14	47	M	+	++	+	+++
15	54	F	+	+++	+	-
16	56	M	+	-	-	-

CASES WITH INHALATION OF GASTRIC CONTENT

1	26	M	+++	++	-	-
2	53	F	+	-	+++	+
3	63	M	+	-	-	++
4	53	F	+	++	++	+
5	71	F	+	+	+++	-
6	13	M	+	+	+++	+
7	65	M	+++	++	++	++
8	33	M	+++	+++	++	-
9	66	M	++	++	++	-

CASES OF HEAD INJURY

1	48	M	+	+++	+	++
2	43	M	++	+++	+	+
3	43	M	++	+++	-	++
4	37	M	++	+++	+	++
5	46	M	+	++	+	+++

MISCELLANEOUS CASES

1	52	M	+++	-	-	+
2	61	M	+++	+	+	+++
3	31	M	+++	++	+	+++

4	5	M	++	+++	+	+
5	51	M	+++	+	++	-
6	59	F	+++	+++	++	-
7	69	F	+++	++	+	-
8	83	F	+++	+	-	-
9	33	M	+++	+	-	++
10	41	M	+	-	-	++
11	34	M	++	+	++	-
12	84	F	+	+	-	-
13	26	M	++	+	-	+
14	39	M	+++	+++	-	-
15	51	F	+	++	+++	+
16	19	M	+	++	+	+
17	3	F	++	+	-	-
18	17	M	+	+	+	+
19	23	F	++	+++	+	+
20	7	M	+	++	+	-
21	9	M	+	+++	++	-
22	55	M	+++	+++	++	+++
23	7W	M	+	++	-	-

miscellaneous.

The lesion to be sought was established and arbitery standard for degree and of severity were set up in order to categorize the main pathological findings.

The qualitative data was presented in table-3. All results were obtained from microscopical examination. Individual cases or group of cases were described and then all the cases were considered as a whole and a generalization drawn which included most of the cases.

3.1. DROWNING DEATHS:

All 47 cases had drowned in fresh water. Death occurred rapidly and in none was any evidence of previous lung disease.

In 9 cases there were free alveolar haemorrhages in the form of scattered foci or patches of different severity.

There were 21 cases of vascular congestion, 4 of them had a severe degree, while 10 and 7 had moderate and mild degrees of congestion respectively.

In 14 cases, neither congestion nor haemorrhage were detected in the lung sections. In 29 the sections contained alveolar phagocytes. These phagocytes or macrophages were large cells each with a small nucleus and ample cytoplasm crammed with golden- brown pigments, mostly haemosidrein and anthracotic dust. These cells were found scattered or in groups within the alveoli. In 7 patients phagocytes were copious, while 11 had a moderate degree and the other 11 had a mild degree.

Forty four cases displayed oedema, varying in distribution and extent. Some areas had microscopic fields without oedema while other adjacent fields showed it. In some areas the oedema seemed to involve a respiratory unit or group of respiratory units. In many instances the stainable oedema fluid appeared at the border of the alveolus and not in the centre. The clear area in the alveoli represented air, as the gross observation had indicated that these lungs contained minute air bubbles.

The oedema fluid in drowning appeared as a pink coagulum.

The degree of oedema ranged from barely detectable to almost complete inundation of most alveoli.It was severe in 8 cases, while in 7 and 29 cases it was moderate and mild respectively. Lung sections in 3 other cases failed to show any oedematous fluid.

Though the oedema appeared in patches it was fairly extensive throughout the lungs.

The peribronchial, perivascular and subpleueral lymphatics in most cases were not dilated. The most noticeable feature in the lung tissue sections of drowned patients was pulmonary oedema. Alveolar phagocytes were found in greater quantity than expected.

There was haemorrhage in 19% of cases and vascular congestion in nearly 44%.

An other pathological feature demonstrated the evidence of emphysema in nearly all the sections of lung. These

changes included overinflation and distension of alveoli with stretching and sometimes destruction of the interalveolar septa. Partial collapse of alveoli were sometimes found in between the overdistended alveoli.

3.2. CARDIAC CASES:

All the 18 victims had died suddenly , in the street, at home or at work. There were 6 cases, with different degrees of alveolar haemorrhage, either diffuse or patchy. Most of cases had sections that were uniform in appearance. In these patients the histological apperance showed that the oedema was mild to moderate, while the congestion was moderate to severe. Alveolar phagocytes ranged from mild to moderate.

In this category of cases, oedema was observed in 18 cases, congestion in 16 and alveolar phagocytes in 10 cases. The most noticeable feature in these cases was the amount of congestion .This was observed in almost every microscopic field and involved all the vessels. The distribution of oedema was not as patchy as in cases of drowning but more diffuse.

The sections showed good preservation of lung structure as neither overdistension of alveoli, with or without oedema, nor rupture of the alveolar septa could be seen.

3.3. CASES OF BRAIN INJURY:

These 5 cases included skull fracture which resulting

from falls, automobile accidents or ruptured aneurysms of cerebral arteries. Most of these deaths occurred in a matter of minutes.

All the cases presented a uniform pattern of mild to moderate oedema, moderate to severe congestion, mild haemorrhage and moderate to severe alveolar phagocytes. Only one case presented without alveolar haemorrhage.

The outstanding features of the brain injury cases were congestion and haemorrhage. In all of them the lung sections showed congestion and 4 revealed haemorrhage. Also, in these cases, there was good preservation of the lung structure without any abnormality in the alveoli.

3.4. MECHANICAL ASPHYXIA:

Sixteen cases under this heading resulted from epilepsy, hanging, manual compression of the neck, traumatic asphyxia and suffocation.

Congestion was the prominent feature in 15 cases and was similar to the findings in cardiac and brain injury deaths. One case presented with severe congestion, six cases showed mild oedema, haemorrhage was present in 11 cases but was mild, and alveolar phagocytes were observed in 8 cases.

The asphyxial cases showed prominent congestion in nearly all the sections of their lungs, and was almost as pronounced as in cardiac and brain injury deaths.

Oedema was variable. Haemorrhage was a distinctive feature in these cases.

No other abnormalities of the alveoli, similar to those seen in some drowning cases, were observed in asphyxial deaths.

4.5. CASES WITH INHALATION OF GASTRIC CONTENT:

All cases were diagnosed on the basis of macroscopical finding of gastric contents in the air passages, as well as on the microscopical appearance of foreign particles. Autolytic changes of the respiratory mucosa were caused by the gastric juice and sometimes the finding of bacterial colonies.

In all 9 cases oedema varied in distribution and extent.

Congestion , haemorrhage and alveolar phagocytes were found in the lung sections of 7, 7 and 6 cases respectively.

The outstanding feature here was oedema with moderate to severe haemorrhage distributed in patches throughout the lung tissue.

Emphysema aquosum was not seen in these sections as no distension of the alveoli nor rupture of alveolar walls were featured.

3.6. MISCELLANEOUS CASES :

For simplicity of pathological description, these cases were categorized under 3 headings including: pulmonary oedema, drug intoxication and other cases.

In the first category the cause of death was acute

pulmonary oedema as no other pathological finding could be found to account for death.

The main pathological feature in these cases was severe and diffuse oedema involving all the alveoli. Congestion was also present in 2 cases in which a severe degree of congestion had been found in 2 of the fatty liver cases.

Tissue sections from the 5 cases showed mild to moderate haemorrhage distributed in a diffuse or patchy pattern. Few to numerous alveolar phagocytes were found in 4 of the cases.

3.7. DRUG AND GAS POISONING:

These included poisoning with alcohol and proparonol, thyophylline, prothiaden, distalgesic, aspirin and carbon monoxide.

These cases are described individually as nearly every case differs from the other , and one may refer to table (Tab.3) for comparison.

The lung section from alcohol and propanolol intoxication presented severe patchy oedema and congestion. No haemorrhage nor alveolar macrophages were found. The lungs in thyophylline poisoning were characterised by mild patchy oedema, moderate congestion and scanty alveolar phagocytes. Alveolar haemorrhage was severe in the one case and mild in the other.

The prothiaden case showed moderate patchy oedema with mild congestion, but no haemorrhage nor alveolar

phagocytes.

In the distalgesic and aspirin case, the lung sections showed oedema, congestion, haemorrhage and scanty alveolar phagocytes.

In carbon monoxide poisoning, the oedema was moderate and distributed in patches over all the sections and was accompanied by severe congestion. There were also haemorrhages and alveolar phagocytes both in a mild degree.

In all the sections there were no structural abnormalities of the alveoli.

In 2 cases of gas explosion, the sections showed mild diffuse oedema with severe congestion but absence of alveolar phagocytes. Haemorrhage was mild and discrete in one case and moderate and diffuse in the other.

In the last category oedema was detected in all, usually in a diffuse pattern, along with mild congestion. In the example of pulmonary thrombo-embolism, both congestion and haemorrhage were absent. However, haemorrhage was the outstanding feature in the shock lung in the case of acute hepatic failure. Slight extravastion of blood was seen in the case of diabetic ketoacidosis but all other cases were negative. Alveolar phagocytes were detected in only 4 cases.

4. DISCUSSION:

Of the 118 cases in this study, oedema was very common in the lung sections. It varied from the barely

detectable to the nearly complete filling of all the alveolar spaces. The appearance of oedema did not usually conform to the classical description , in which the fluid is considered to be an homogeneous, pink staining, cell-free coagulum completely filling all the alveolar spaces.

A granular appearance of the oedematous fluid was described in drowning (61) which indicated the presence of globulin. The lungs of drowning victims are not infrequently filled with a high-protein oedema fluid. Under normal conditions, a small quantity of fluid and protein enter the perimicrovascular space of the lung, and are removed by the pulmonary lymphatic system. Oedema results when the volume of the fluid entering the pulmonary perimicrovascular space exceeds the capacity of the lymphatic system. Fluid expands the perimicrovascular space and eventually overflows into the pulmonary alveolus.

Permeability pulmonary oedema has been invoked in certain of our cases including the aspiration of gastric content, shock, drug overdose and pulmonary embolism.

The results of the microscopic observation on the lung tissue sections from human cases indicated that pulmonary oedema occured in a variety of sudden deaths, including drowning. They could not be differentiated one from another.

Pulmonary oedema in fresh water drowning is thought to result from chemical and physical changes of the alveolar surface, and from the circulatory overload caused by the

inhalation of water. It was considered as the main cause of death in both types of drowning.

In cardiovascular disease, the oedema is caused by increased pulmonary capillary pressure which differs from the mechanism of oedema operating in drowning, though both types nearly indistinguishable.

In brain injury, neurogenic oedema with severe congestion was described long ago,yet its precise cause is still uncertain.

Oedema with prominent congestion was also reported in cases of strangulation.

The results from this study show that oedema was present at necropsy in most cases of sudden death. Also the presence of other morbid features e.g. congestion, haemorrhage and alveolar phagocytes are so common that it is very difficult to rely upon histological examination of lung tissue in the diagnosis of drowning.

However dilatation of the alveoli with or without oedema, and rupture of the alveolar walls has been seen frequently in the lungs from drowning cases. Even though it cannot be considered as a sign for diagnosis of drowning. Alveolar phagocytes or desqumated epithelial cells were found in almost every sudden asphyxial death including drowning.

The oedema fluid, as seen in most cases of drowning, was distributed in a patchy pattern. In drowning acute oedema of the lung is a rather explosive process, the

fluid enters the alveoli from vessels, quickly filling them and then pouring out into the alveolar duct.If the adjacent primary lobule does not contain oedema, the air in this lobule might be trapped by the fluid. Since oedema fluid has a high surface tension, it has the ability to trap the air. Thus air in some areas, and fluid in others, account for the patchy distribution. That is why air trapping which appears usually on the true surface of the lung, is considered as a distinctive postmortem feature in drowning. The oedema in drowning differs from that of cardiac failure, brain injury and strangulation, where the whole complex of haemodynamics within the pulmonary circulation is undergoing an irreversible transit from the blood stream to the alveolar spaces. Drowning results in filling of the lung with a liquid which by definition is a form of pulmonary oedema.The water must move first into the blood before the blood elements move into the alveolar spaces. As a result fresh water drowning causes movement of water into the vascular compartment, but sea water drowning produces a loss of water from the vascular compartment.

Lungs of drowned persons are not infrequently filled with a high protein oedema fluid, suggesting an alteration of pulmonary capillary permeability as being produced by osmotic damage. Since congestion occurred in nearly half of the drowning cases, the oedema formation may involve some degree of a vascular haemodyanmic complication.

5. CONCLUSION:

The study of lung sections of 118 cases with varying causes of death, including 47 of drowning, was made to establish the histopathological findings, in order to assess the reliability of histological examination in the diagnosis of drowning.

The series included deaths from cardiac failure, brain injury, strangulation, suffocation, hanging, aspiration of gastric content, fatty liver, diabetic ketoacidosis, cot death, peritonitis, pulmonary thromboembolism, gas explosion, alcohol intoxication, drug poisoning and carbon monoxide poisoning.

It transpired that it was difficult to differentiate cases of drowning histologically from other sudden asphyxial deaths,though each type of death had minor distinguishing features. For example, congestion was prominent in asphyxia, cardiac failure and brain injury, haemorrhage was a marked feature of cases of brain injury, and oedema was the outstanding feature of drowning and was usually unaccompanied by haemorrhage.

Other pathological findings in drowning were the distension of the alveoli with stretching of the alveolar walls. Other alveoli which were free from oedema fluid, were either distended by trapped air or collapsed. These features however are not specific and frequently found in cases of drowning.

Generally, little reliance can be put on histological

examination of lung tissue in the diagnosis of drowning, as most sudden asphyxial deaths share the same pathological features.

SECTION 2

ASSESSMENT OF LUNG WEIGHTS IN DROWNING CASES:

1. INTRODUCTION:

In order to obtain a clear picture of a drowning case, the weight of the lung should be measured carefully . In forensic pathology, it is not uncommon to find a dry lung, and yet unmistakably the case was one of drowning. It is also important where a body is found in water, to decide if drowning is really the cause of death. Therefore this study has been conducted to analyse the weights of the lungs in cases of drowning, and compare them with the lungs from other causes of death.

2. MATERIALS AND METHODS:

The investigation was carried on those cases of death which occured by violent, unnatural or unexpected means. For this study a group of 50 natural (age 18 years and over) were collected and analysed as to age, sex, right and left lung weights. For this group all decomposed , hospital-delayed deaths, and those in which intrinsic lung pathology was present (e.g. pneumonia, cancer) were excluded.

A second group of 50 normals (age 14 and over) were collected of sudden type of deaths without apparent abnormality of the lungs. They were likewise analysed.

Finally, 38 fresh water drowning deaths were chosen

from those cases which been recovered from the water. Also all decomposed or those with coexistent lung pathology cases were excluded. The 38 fresh water drownings were studied and compared with the 'natural' group and the 'normal' group.

The natural sample consisted of a group of 30 males, and 20 females, whose ages ranged from 18-79 years, where the cause of death was atherosclerosis or acute alcohol intoxication.

The 'normal' sample comprised 31 males and 19 females with an age range of 14-89 years, the cause of death being road traffic accidents, stab wounds to the hearts and cerebral haemorrhage.

3. DISCUSSION:

The question of 'normal' lung weight must be addressed. Certainly the natural population are not normal, for being older with some degree of atherosclerosis, there may well be some degree of pulmonary oedema. Probably the true normal lung weight might be found with causes of death which are totally unrelated to the lung. Therefore we selected cases whose lungs appeared normal at necropsy and died suddenly from causes unrelated to the lungs. After answering the question related to 'normal lung', the measurement of weights in drowning cases have to be analysed carefully to know the meaning of 'dry lung'.

Tables 4,5,6 summarised the descriptive analysis of lung weights in normal, natural and drowning cases

respectively. It shows that the weight of the left lungs ranged from 250-900 gm.with an average value from 534.9 gm.; while the figures for the right lungs are 350-1200 gm. with an average value from 639.7 gm. The standard deviation of the weight of left and right lungs are 161.3 and 193.9 respectively.

Table-7 addresses itself to the problem of 'dry' lung in drowning. If one uses the table-1 as the hypothetical 'normal' weight and uses this as a 'cut off', then in table-4 all the cases equal to or below 300-400 gm. are 'dry'. This dry percentage in fresh water cases ranges from approximtally 22.8% for the left lung and 8.5% for the right lung. These figures show that much care should be paid to the dry lung phenomenon, especially where the circumstances are unknown.

Next, one may wish to know how the fresh water drowning cases statistically compare with 'normals' or 'naturals'.

Therefore, t-test was performed in comparing fresh water drowning lung weights to 'normals' (Tab.10) or 'naturals' (Tab.8). There are statistically significant differences. Also, the natural population lung weights are statistically significantly different from the normal lung weights (Tab.9).

4. CONCLUSION:

Generally, several useful conclusions can be reached concerning lung weights in drowning cases. One of these

TABLE-4

Descriptive analysis of ' normal' lung weights

Sample size N = 50

Weight	Left lung	Right lung
Minimum	180	200
Maximum	480	525
Range	300	325
Median	340	400
Mean	328.8	379.9
S.D.	69.1	78.7

TABLE-5

Descriptive analysis of 'natural' lung weights.

Sample size N = 50

Weight	Left lung	Right lung
Minimum	300	400
Maximum	1000	1200
Range	700	800
Median	600	750
Mean	624.0	739.9
S.D.	187.4	189.6

TABLE-6

Descriptive analysis lung weights in fresh water drowning

Sample size N= 35

Weight	Left lung	Right lung
Minimum	250	350
Maximum	900	1200
Range	650	850
Median	500	600
Mean	534.9	639.7
S.D.	161.3	193.9

TABLE-7

Frequency of distribution of lung weights in Fresh Water Drowning.

Weight	Left lung (% of cases)	Right lung (% of cases)
< 200 g	0	0
200-300 g	5.7%	0
300-400 g	17.1%	8.5%
400-500 g	25.7%	25.7%
500-600 g	20.0%	22.8%
600-700 g	11.4%	14.2%
700-800 g	14.2%	17.1%
800-900 g	2.8%	2.8%
900-1000 g	0	8.5%
> 1000 g	0	2.8%

TABLE-8

STUDENT t-TEST

Fresh water drowning vs."natural"

	Left lung/drowning	Right lung/natural
Mean	534.9	624.0
S.D.	161.3	187.4
Observation	35	50
t-test	2.3	
D.F	83	

Degree of significace 0.025

	Right lung/drowning	Rightlung/natural
Mean	639.7	739.9
S.D	193.9	189.6
Observation	35	50
t-test	2.4	
D.F.	83	

Degree of significace 0.020

TABLE-9

STUDENT t-TEST

"Normals" vs."Naturals"

	Left lung/normal	Left lung/natural
Mean	328.8	624.0
S.D.	69.1	187.4
Observation	50	50
t-test	10.4	
D.F.	98	
Significance	0.000	

	Right lung/normal	Right lung/natural
Mean	379.9	739.9
S.D.	78.7	189.6
Observation	50	50
t-test	12.4	
D.F	98	
Significance	0.000	

TABLE-10

STUDENT t-TEST

A. Fresh water vs. "normals"

	Left lung/drowning	left lung/normals
Mean	534.9	328.8
S.D.	161.3	69.1
Observation	35	50
t-test	8.1	
D.F.	83	

Degree of significace 0.0001

	Right lung/drowning	left lung/normal
Mean	639.7	379.9
S.D.	193.9	78.7
Observation	35	50
t-test	8.5	
D.F.	83	

Degree of significace 0.0001

is that approximately 80-90% of fresh water drowning cases have 'wet' lungs. The second conclusion is the presence of a significant difference, statistically, between lung weights of fresh water drownings and the natural or normal population.

CHAPTER TWO

THE SIGNIFICANCE OF THE DIATOM TEST IN THE DIAGNOSIS OF DEATH BY DROWNING

1.INTRODUCTION AND LITERATURE REVIEW :

1.1 DEFINITION AND DISTRIBUTION OF DIATOMS

Diatoms are members of the class of minute unicellular plants called bacillarriophycea. They are encountered in the particulate matter called plankton which consist of algae, diatoms and a variety of debris found in water (174).

They are essentially unicellular algae, although chains of cells and colonial aggregates may occur. Many are supplied with a delicate and beautiful heat- and acid-resistant siliceous skeleton which explains the presence of diatoms in the fossil record. However, the degree of silicification varies from very heavy to extremely light. Marine diatoms, for instance, are more fragile than their fresh water counterparts.

There are some 15,000 species of diatoms (76), both living and fossil. In British coastal waters alone there are 771 species in 104 genera of diatoms (75). The diatoms vary in size between 2μ-1mm in diameter, but most are from 10-80μ and up to 10 mm in width, if they are elongated (76).

By the slow accumulation of dead cells on the floor of

lakes and the sea, a deposit of diatomaceous earth is formed which is called kieselguhr. Diatoms are usually, though not always, present in natural water. They are found wherever there is water and sufficient light to support photosynthesis. They are abundant in ponds and often occur in unpolluted water (174). Roughly half of them live in fresh water while the remainder live in the sea or in brackish water (76).

The systematic classification of diatoms is based on the structure of the siliceous valves. The valve is referred to one face of the outer shell of the diatom, which is called the frustule. This is usually made up of two valves each with a surrounding "girdle" joining the two valves. Also the presence of a cleft or slit in the middle of the valve on the apical axis of some elongated forms, is considered an important criterion for classification. This cleft is called a "raphe" which is an opening fissure running along the apical axis of the diatom, permitting the cell content to have contact with the external medium in which it lives (76). However there is some difficulty in the identification of diatoms, which proposed to classify them roughly into two ill-defined groups (76). The first, the pennate diatoms, whose valves are elongated or linear, usually have a raphe and have their puncta or costa arranged symmetrically on either side of the raphe. The second, the centric diatom in which their valves are usually circular, triangular or polygonal in shape. These have no

raphe and have the costa or puncta arranged radially about a central point. However, many diatoms do not fit into this scheme of classification, which leads to further attempts to formulate more comprehensive schemes (75). Not withstanding, the valves provide a vast array of indestructible siliceous element of microscopic size, highly characteristic in shape and form, and therefore easily recognizable (76).

Diatomaceous earth consists mainly of amorphous silica which has high chemical and thermal resistance properties which has led to the wide spread use of this product for insulation , as an absorbent for chemicals , as a carrier of catalysts, filters, additives to cements, paints, paper asphalt, rubber and other products.

1.2 METHODS USED FOR EXTRACTION OF DIATOMS. REVIEW:

Several methods have been used for the extraction of diatoms from different types of human tissue (82,89,124,176). Maceration of the lung tissue is an early technique (138) which affords reliability and speed, together with showing green algae when they are present. In modifying this method, some workers digested lung tissue with strong acids and looked for diatoms in the residue (138). Although this method allows recovery of all the acid-resistant diatoms, the green algae and some fragile diatoms are lost (174). These chemical methods, however, are not recommended by some workers

(39), because of the high possibility of contamination and also they do not permit exact histological location of diatoms. However, the chemical methods which have been used for digestion of bone marrow (168,171,175), provide most helpful results when only bones are available. An alternative procedure is by the incineration of bone marrow using an electrical furnace to get rid of the fat usually present (174).

Other workers have examined the tissue histologically in dark field microscopy, the refractive index of which has been changed by replacing xylol with mono-bromobenzol, where diatoms stand out like tiny spots of light against dark background (82).

Another method of lung digestion was suggested by using a strong base or digestive enzymes such as pepsin (124). Also tissue solubilizer such as Solune was used for the digestion of lung tissue, which was enhanced by utilizing ultrasonic waves to speed up the dissolution of the tissue (55).

1.3.DIATOM AND THE LUNG:

A major advance in the practice of forensic medicine was achieved by demonstrating the particulate matter in the lung tissue of a person drowned in the water containing these particles (139). That particulate matter is called plankton, which consists of algae, diatoms and a variety of microscopic debris. However, the

significance of this discovery has been casted aside by the demonstration of diatoms penetrating the alveolar capillaries and then to be found in the internal organs (81,117). Further more, the reliability of the lung, as an organ for the demonstration of diatoms, has been challenged (117,145,176). One reason for this criticism is the possibility of penetration of diatoms postmortem (39,117,145,176). The main criticism, however, directed against the reliability of finding diatoms in the lung, was that diatoms could be found in cases when there was no question of drowning (22,58,97,126,151,154,155,175). For example workers in the kieselguhr industry, had shown diatoms loaded in their lungs without, of course, having drowned (126). The diatoms had also been detected in the lung of non-drowned subjects who had never been in direct contact with these particles in their lives (93,133). On account of the frequent presence of diatoms in the lung of non-drowned subjects, the lung has therefore been considered as a controversial organ for such a test (22). Accordingly, the finding of diatoms in the lung tissue is considered as unreliable and an unspecific phenomenon (137). This view has been based on the idea that the presence of diatoms in the lungs has been consistently found in cases of non-drowning subjects. However, such a view did not remain without a challenge from other workers who believed in a contrary opinion to that (117). In a series of 16 cases of drowning they all showed diatoms in the lungs, while 19 out of 24 cases of non-

drowning deaths were negative (174). The positive results in the other 5 cases were due to contamination by exhumation, and immersion in water after death. Nevertheless, there is agreement that the finding of a very few diatoms e.g. 1-3 per 100 gm of lung in non-drowning deaths, is not considered enough reason for abandoning the lung as a subject for study (55,61,168,171,175). This view has been confirmed by the examination of 15 control cases which showed an absence of diatoms in the lungs whilst positive results were obtained in 36 out of 43 drowning cases (121).

Other workers interpreted the positive findings of diatoms in the lungs as indication of drowning only when they are found beneath the pulmonary pleura (176). Nevertheless, an extreme view has been held, that in areas where no diatoms could be found in the air, the negative findings of diatoms in the lung of immersed bodies suggests that the death was not due to true drowning (121).

Generally, most of the authors and workers in this field have agreed that lung examination alone is insufficient, and that other systemic organs should be involved for more conclusive evidence in order to lessen the doubts about the possibility of entrance of diatoms into the lung antemortem or postmortem (55,61,87,168,174,175).

1.4 DIATOM PENETRATION INTO SYSTEMIC CIRCULATION:

According to the early work on drowning in the 19th. century,the penetration of water with a variety of suspended microscopic debris into the minute ramification of air passages, and the absorption of inhaled water into the circulation was demonstrated experimentally to occur if the period of submersion was 20 minutes or more (17). However in recent studies the absorption of water into the circulation was found to occur more rapidly than the above mentioned time, as 50% of the circulating blood was found to be diluted after only 3 minutes of submersion (163). In 1888 (127) Prussian Blue, a tracer material , was observed to penetrate the lung capillaries into the left side of the heart. After the discovery of diatoms in the fluid from dissected lung tissue, it became possible for the first time to surmise that diatoms entered the systemic circulation from the lung, although they had not been shown in the blood of these cases (138). This finding refuted the idea that plankton are filtered out in the pulmonary alveoli. This discovery was to prove fruitful, in the long run. The mode of transport of diatoms into the systemic circulation through the lung has been confirmed by other workers (81,169,176). Not only diatoms, but also pulmonary alveolar macrophages and crystalline artefact particles have been detected in the blood of the left side of the heart, as they migrate from

the lung into the circulatory system during the process of drowning (81,169,176). As the pulmonary capillary pressure is lower than other parts in the lung and the velocity of blood in the lung is highest when the alveoli are distended, the absorption into the blood is explained according to the extreme decline in intravascular pressure between alveoli and the left ventricle, which in the course of drowning leads to a strong negative pressure which induces the indrawing of diatoms into the blood from the distended alveoli and ruptured pulmonary vessels (176). Furthermore, the tear of the pulmonary alveolar wall is believed to be a prerequisite for the passage of diatoms into the circulation. Forceful respiration against a closed airway was demonstrated experimentally to imitate respiration against a closed glottis which occur in drowning, thus rendering laceration of the alveolar walls more likely (154). However, it was found to be only a small number of diatoms which could penetrate into the lung capillaries in order to reach the left heart from where they were dispersed all over the body (58). Such distribution was observed by using P^{23} as a tracer element for drowning water which showed 3/5 of the element to be concentrated in the lung, and about 2/5 in all other parts of the body (176). A similar kind of distribution has been observed by using other indicators such as china ink, Sudan III, diatoms, Ca^{45}, I^{131}- human serum albumin (169). However, diluted tritrium- water was preferred because of a lesser

affinity towards the organs and a tissue. The distribution of these materials generally in the systemic organs was found to be as follows; lung, blood of the left side of the heart, heart muscle, blood of the right side of the heart, liver, brain, pancreas, spleen, kidney, skeletal muscle, bone, seminal vesicle and testis (169). Other experiments were conducted on rats drowned in a suspension of kieselguhr neutron activated, for 24 hours. After 15 minutes they were examined with a sensitive X-ray plate for a period of 12 days. The most pronounced radioactivity was detected in the lung and heart whilst other organs showed decreasing radioactivity in the following order, liver, left kidney, right kidney and spleen. The idea of penetration through the lungs is supported by the finding of diatoms in the organs of persons who had been in contact with these particles during their lives.

The other possible route of absorption is thought to be through the gastrointestinal tract (151,154,174). It was shown that the particulate matter could pass from the gut via the lymphatic vessels and the thoracic duct into the blood stream. This was confirmed experimentally, by feeding rats on a watery suspension of diatomaceous earth which was then discovered in nearly all their organs (155). An emphasis on the absorption through the gastrointestinal tract was based on the finding of large size diatoms in the systemic organs, which could not have been passed through the alveolar wall which has a

permeability limit of 10-12μ (155). Others believe that the maximum diameter of diatoms able to reach the circulation via the lung is about 30μ (176), but even this seems rather large in view of the diameter of the pulmonary arteries. In order to study the absorption through gastrointestinal tract, rats were fed on a suspension of kieselguhr orally and by a duodenal catheter (174). At the end of 24 hours the elimination of the diatoms was virtually complete, with only 1% of diatoms still remaining in the gut, and approximately 2.5%, 1.2-1.4%, 80-90% and 2% of diatoms being eliminated from the bile, lymph, feces and urine respectively.

The difference in the ability of both the lung and gastrointestinal tract to absorb different diameters and sizes of diatoms has been studied experimentally by using two sets of animals. One set was fed on kieselguhr in gelatin capsules, whilst the other was designed to inhale a suspension of kieselguhr in water (155). The diatoms were discovered in the blood of the both group with diameters of 30-80μ in the first groups and 12-14μ in the second group. These results supported the idea that more diatoms are discovered following ingestion than inhalation where the lung acts as a filter. This opinion, however was contested by the failure to detect diatoms in the organs of a body found where the drinking water contained great quantities of diatoms (133).

1.5 DIATOMS AND SYSTEMIC ORGANS:

The demonstration of diatoms in the systemic circulation was an important discovery in the field of legal medicine, it made dramatic progress in the diagnosis of drowning, by linking the inhalation of water with the finding of diatoms in the systemic organs.

The better understanding and reliance of this method of the diagnosis of drowning was based almost entirely on the studies of non-drowning cases (58,97). The negative results obtained from these cases substantiated the validity of the diatoms test as a reliable method for the diagnosis of drowning. This view was confirmed by negative results obtained from cases removed from water(131). However, if the finding of diatoms was found only in the human organs from drowning, the test then would be the answer to many of the difficulties related to the diagnosis, but unfortunatly this is not always the case (58,97,126,151).

Experimentally, rats which were fed on kieselguhr supplement, showed diatoms in 92% of systemic organs, but more surprising results were the finding of diatoms in the internal organs of 49% control animals not fed on the supplement (151). Also, while other workers tried to discover experimentally, a special species of diatoms in drowning fluid, they found co-incidentally a variety of diatoms virtually not present in the original experimental culture (155). Nevertheless, the criticism against the reliability of the diatom test was

substantiated by the finding of diatoms in the organs of non-drowned subjects (131,151). For example, results from the livers of 22 non-drowning corpses disclosed various forms of well preserved and typically structured diatoms in 21 cases with numbers ranging from 1-111. Further evidence of the accidental finding of diatoms in the systemic organs came after the examination of 20 non-drowned cases in which 50% and 40% of them contained diatoms in the liver and kidney respectively. These results are confirmed by positive results obtained from non-drowning cases, conducted by other workers (131,133). These results, in general, lead to the belief that identification of diatoms in liver, spleen and kidney must be regarded as equivocal. The critics, also, built their challenge on the basis of finding diatoms in the organs of children, still-births and premature infants dying from causes other than drowning (58). It is thought that the origin of these diatoms was due to the contamination of the food, or during the process of the disorganization technique used for the detection of diatoms.

Therefore, according to the number of diatoms usually discovered in the liver of non-drowning cases, the detection of diatoms permits the diagnosis of drowning being justified only when the number discovered from the liver is high. In other organs like heart and brain, never more than a very few number were which is thought to be in sufficient in establishing the diagnosis of

drowning (172).

Generally, however, there is a wide agreement about the sufficiency in the detection of diatoms in the systemic organs, as a supportive evidence of the diagnosis of drowning.

1.6. BONE MARROW AND DIATOMS:

It was a significant advance in medico-legal science when bone marrow examination was used as a confirmatory diagnostic method in cases of drowning (168). It is thought to be the most applicable internal tissue for that purpose. A conclusion was reached, that not only the presence of diatoms in the bone marrow indicates death by drowning, but that the exclusion of drowning could also be based on the negative results obtained from that tissue. Such a conclusion resulted from the failure of finding diatoms in the bone marrow of 7 subjects who were shot before entering the water (175). The reliability of bone marrow is also emphasized by other workers (76,175), to the extent of the diagnosis of drowning being determined by the presence of a single diatom in the bone marrow (175).

The determination of drowning as a cause of death by the examination of the bone marrow for diatoms, has been reported by many authors (97,168,171). On the other hand, diatoms have been detected in non-drowning cases (133), and such observations have been supported

experimentally (87,97). In one study of 35 non-drowning cases, the identification of diatoms was made in 12 of them. These results were supported by an other study where positive results had been obtained from 15 out of 16 non-drowning cases. The importance therefore of a full examination of control cases is emphasized to assess the reliability of bone marrow for the examination of diatoms. Also, other investigations were made to study the possibility of finding diatoms in the bone marrow of non-drowned bodies which were buried under the ground, or immersed in water for a period of time ranging from 1 day to 1 month (97). These studies, however, failed to show any diatoms in the bone marrow. In the same series, a comparison was made between unbleached fresh bones and completely bleached bones after both types being immersed in a diatom-containing water. This showed an absence of diatoms in the first group of bones whilst diatoms were present in the second group, after 1 day immersion in the water. Attempts were also made to find a relationship between the depth of the water, and the penetration of the diatoms into the bone marrow using the bleached bones of rabbits (87). Diatoms were detected in a few bones which had been immersed in shallow water but in all bones, that been sunk to a depth of 30 metres and more.

From the above observation, an error in the determination of drowning may be predicted especially in skeletonized bodies which have been submerged for a length of time and when the possibility of intrusion of

diatoms could be made through the foramen nutricium and other pores in bone. Although, most authors are convinced that only small number of diatoms are usually discovered in bone marrow (171,174), others insist that the number of diatoms present should be taken into consideration before any conclusion should be given.

Generally, the bone marrow is advocated as the most suitable tissue for the diatom test (171,172,174,175), in which the contamination can easily be avoided.

1.7. POST-MORTEM PENETRATION OF DIATOMS INTO THE BODY:

The credibility of the diatom test as a reliable means of the diagnosis of drowning was challenged by raising the possibility of intrusion of diatoms post-mortem. Intrusion of water into the lung of an immersed body is a recognized phenomenon, even before the discovery was made of diatoms in the lung (174).

Some workers are keen to demonstrate that life was still present from the finding of microscopic plants, animal remains, silt, coal dust and other particulate matter in the alveoli (62,147). This vital phenomenon of the presence of artefacts in the alveoli, was concluded from the explanation that only in the presence of respiratory movement can these particles be carried into the depth of the lung. However, other workers have denied this (117,172). Although the possibility of the intrusion of foreign matter into the lung after death might be

small in amount and limited in distribution, it will therefore reduce the value of the diatom test. This hard view about the diatom test has been rejected by demonstrating that water and solid substances including diatoms did not reach the periphery of the lung except in rare cases (176). Nevertheless, only in the case of general dissemination and uniform distribution of foreign particles throughout the lung can be considered as a vital phenomenon (76). Similar results have been obtained from human bodies who have been subjected to a high hydrostatic pressure by prolonged immersion at a considerable depth of water. It was claimed that under hydrostatic pressure, diatom intrusion did not occur into the lung only, but also into other internal organs as well. Diatoms could even be discovered in liver, kidney and other organs in bodies which had been immersed to a depth of 130 metres (176). However, there is no shared agreement between most of the authors about the possibility of penetration of diatoms into the systemic organs after death.

1.8. CONTAMINATION WITH DIATOMS :

The finding of diatoms in the organs of non-drowning cases has directed the attention towards the search for the origin of these particles. After extensive studies in this field it is contested that diatoms are present everywhere, and that contamination with these particles

is nearly unavoidable (97,126,133). Air pollution, for example, was demonstrated by using an air filtration band in which large number of diatoms had been discovered. In an other study, the air of a city was shown to contain a large number of diatoms which were similar to the species found in the river of that city (151). To refute this objection, the experiments were repeated on 13 other cities in which large numbers of diatoms had been detected in the air (58). For that reason, it is alleged that a person living in those cities is liable to inhale around 15 diatoms a day.

The view of air contamination was confirmed through results obtained from the lungs of non-drowned cases, especially in workers who were exposed to contamination by diatoms through mining, and the production and application of diatomaceous earth (126). The number of diatoms in the air however varies from one area to another, as is shown by some studies (38).

An other possible origin of diatoms is the contamination of fruits and vegetables (151,154,155). For that reason examination of water in the stomach was omitted in cases of drowning because of the possible contamination by the ingestion of unwashed food.

Diatoms are also contaminants in tap water (166), which could be the explanation of positive findings in cases of non-drowning, as well as of contamination of materials used in laboratories. Even in distilled water, bi and tri-distilled water, diatoms were still being discovered

(58). These types of solutions are however considered safe from contamination.

Apart from all those proven sources of contamination, there is one other, which is the most difficult to quantify and that is the contamination of glasswear and reagents (97,117,126,133,155).

2. MATERIALS AND METHODS:

In order to have enough cases for examination, specimens were taken from all the bodies recovered from water, and from those subjects which had died from natural and unnatural causes other than drowning. They included 63 cases which showed classical signs of inhalation of water. They are incluided in the group of typical drownings. There were 3 other cases diagnosed as immersion deaths. The diagnosis in the other 4 cases recovered from water, was made on the presence of causes, other than drowning and immersion. The specimens were collected from late 1985 until early 1988 and covered all the cases of submersion in the Glasgow area. These comprised of bodies recovered from rivers, the sea, lochs, canals, ditches, bathtubs and swimming pools. Sixty three cases of drowning were distributed from January to December and in the following manner, respectively,4, 3, 4, 6, 5, 11, 1, 5, 4, 5, 4, and 3. The 3 cases of immersion were encountered in March, September and December whilst the 4 cases of submersion were discovered in June, February, March and April. The bulk

number of drowning cases occurred in the River Clyde.

In order to assess the reliability of the diatom test and to investigate the degree of contamination of human organs with the diatoms, 75 control cases have been examined according to the same rules of examination of the drowning cases. These cases include sudden death due to a variety of causes, mainly sudden unexpected death due to cardiac diseases, respiratory illness, brain injures and asphyxia, especially hanging. Bone marrow and lung tissue were examined in all these cases.

2.1. METHODS:

From both groups of cases, specimens of lungs and bone marrow were examined for diatoms. Six small segments, each the size of sugar lump, were cut from each lung, 2 segments being from each lobe of the lung. The segments were cut from just below the pleural surface making the cut as peripheral as possible, yet at the same time avoiding any possibility of contamination from handling the outer surface of the lung. Each segment was placed in a clean plastic container and processed the same day.

2.2. PROCEDURE:

2.2.1.LUNG TISSUE

From each segment, 5 gms of tissue was processed using a very strict cleaning procedure for the cutting and handling of the specimens. Each segment was cut into

several small pieces using a very clean knife. Each gm. of tissue needed to be macerated in 1.5 mmol/l of pH 10.5 "tris" base, which was prepared by adding 121.14 gram of Tris base into 1 litre of distilled water. The mixture of tissue and Tris solution, was then incubated at 60 C° for 1 hour with 10 milligram of subtilisin-A.

Subtilisin-A (Novo enzymes product, Windsor, U.K.) is a nonspecific proteolytic enzyme, subtilopeptidase, type carlsberg. It is prepared by adding a capsule which contains 10 mg of enzyme powder into 1 ml of tris base. At the same time, 1 ml of blood stained fluid which had escaped from the lung tissue into polythene container, was incubated with 1 ml of Tris base and 10 mg of subtilisin-A. The solution was then cooled at room temperature and then made up to a volume of 300 ml, adding double distilled water to enhance filtration. At the same time a segment of 5 gm weight, being left from each lobe of the lung, was processed according to the direct method. According to this direct examination technique, 5 gm of lung tissue was left over night in 300ml distilled water at room temperature, and after energetic shaking the macerated fluid was filtered.

2.2.2. MILLIPORE FILTER:

Millipore filters are characterised by small diameter and a composition of certain materials that make them almost completely resistant to strong acid and alkali. There are different types, depending on the porosity

which ranges from 0.01μ to 14μ.

We used two different types of membrane filter with diameters of 25 mm and 47 mm. These two types have different porosity sizes of 0.45 μm and 1.0 μm respectively. The filter paper of 1.0 μm size was used because the vast majority of diatoms are greater than this size. The second type filter paper of 0.45 μm pore size was used with all the cases in order to check the result obtained from using the first size, and also to detect any diatom with a smaller diameter, that might have been missed if their size is smaller than 1.0 μm.

The filter paper is white and opaque, but with a unique property of becoming transparent if xylol is dropped on it, thus making it possible to look at it microscopically.

The assessment of the approximate number of diatoms was not difficult but tedious, since the counting was required to cover every field of the slide using a high magnification.

2.2.3. FILTER HOLDER:

Two types of filter holder were used, large and small. The large sagittarius type consists essentially of two plastic containers, one on top of the other and joined by a narrow neck in which the filter paper is placed. The small one is of a metal variety, specially designed for small millipore filter papers.It has a syringe attachment which enables the fluid to be filtered under pressure.

2.2.4. FILTRATION:

By using both the filter papers and both the holders, it was possible to filter most of the fluid. Usually all the fluid in the container which contained 30 ml, had to be filtered. The other reason for using both filter holders was that the filter paper often became clogged before the whole volume had passed through. We therefore used all the macerated fluid obtained from the digestion of the lung tissue, in filtration. The filter paper was then removed and placed under a light bulb for 20-30 minutes after being placed on a clean slide. After that a few drops of xylol or toulene were dropped on the paper or the paper itself was soaked in a plate containing xylol. The transparent filter paper is now, mounted with a cover slip and is examined directly under the microscope.

2.2.5. PROCEDURES OF BONE MARROW EXTRACTION :

We decided to use sternal bone marrow to avoid further mutilation of the body by trying to remove other bones like the tibia or femur. The sternum is already removed at necropsy. The marrow of the sternum is usually abundant and free from contamination like other marrow enclosed by bone. Also the sternum can be removed at autopsy and examined later on in cleaner conditions.

Therefore, two techniques for the extraction of bone

marrow were used, depending on the place chosen which was either, the mortuary or the laboratory of the Department.

At the beginning the materials were transferred to the Department where cleaner conditions were available. Each sternum was put in a clean polythene plastic bag either to be processed the same day, as in most of the cases of drowning or to be left in the deep freeze and then examined at leisure. Initially, the following method was used. The sternum with the attached costal cartilages was placed on a board with the internal surface upwards. A longitudinal strip of periosteum, 10mm x 30 mm, was delineated with a knife and removed with the aid of forceps. A rectangle of a slightly smaller size was next marked with a chisel in the bone itself and the lid of the bone removed. The marrow was then collected with a curette. The other method in the laboratory of the department, was to clean the sternum with distilled water, and saw the sternum longitudinally into two halves. With a metal spatula and a pair of strong forceps the marrow was scooped out and placed in a clean beaker.

The second technique used in the mortuary was simple and reliable, provided that the strict clean conditions were applied. The cleaning of the sternum with distilled water was avoided by this method, as any aqueous solution was to be. avoided as much as possible. Therefore we used to clean the blade of 'deseutor' saw as well as the knives and the forceps used for extraction of the bone marrow with distilled water. The sternum then placed on a

board, and by 'deseutor' saw, a longitudinal cut was made in+ the sternum. After that a large quantity of bone marrow which being so exposed and easily extractable, could be collected by using clean forceps.

2.2.6.INCINERATION:

The amount of bone marrow able to be extracted, varied from a teaspoonful to a tablespoonful, but usually an average of 10 gm from each case was submitted for processing using the incineration method. Before submitting the sample for incineration, the bone marrow was placed in highly heat-resistant containers, i.e. siliceous basins, and exposed to direct heat from bunsen burner. This is called the pre-ashing stage which proceed the method of ashing the bone marrow by the electric furnace. The pre-ashing step of burning was useful in getting rid of the organic materials of the bone marrow in order to enhance and quick the process of incineration. Also, it was useful in avoiding the accumulation of smoke inside the furnace. The desired degree of temperature in the furnace, usually 350-400 C°, required approximately 2 hours to be achieved. This degree of temperature was sufficient to ash any quantity of bone marrow in about 2 hours time.

To dissolve the content of bone marrow, the ash was treated with a few drops of the concentrated hydrochloric acid in which the solution turned into a deep to bright yellow colour. After filtration, the filters were dried,

cleared and mounted as before.

2.2.7. WATER SAMPLES:

It is a worthwhile test to examine the water taken from the site of the drowning. The first reason for that is to check that diatoms are present in the water before searching for them in the body tissue, otherwise there is no point of examination of the tissue for diatoms, while they are not available in the water. The second reason is to note the relative proportion of different types of diatoms in the water and to compare those found in the body. Approximately 1-2 litres of water was collected from the different locations using plastic buckets. This method is easy, simple and reliable at least for our type of work. Not all the locations of drowning have been examined for diatoms in the water because of the difficulty in involving other parties who were in charge of the investigations of these cases, especially the police. Therefore, We started collecting water from different loci of drownings, mainly those of the River Clyde, where most of the drownings had taken place. 100 ml. of water, after being stirred several times, was filtered using a 1.0 u porosity millipore filter paper. Each was then dried carefully under a light bulb for about 10 minutes. It was then cleared with xylol, placed on a glass slide, mounted in D.P.X. with a cover slip and examined microscopically. Because of the large diameter

of the filter paper, it was necessary to mount it on a large slide, or to cut it into 3-4 parts and then examine it on an ordinary slides.

2.2.8. EXAMINATION OF CONTROL MATERIALS FOR DIATOMS:

In order to avoid any source of contamination with diatoms, the materials used in the technique of the diatom test were examined. They included tap water, distilled water, xylol, toulene, hydrochloric acid, filter paper and air. Half a litre of each of the fluids was filtered, cleared and mounted in the usual manner. For air filtration we used a large millipore filter which was laid on one of the benches in the laboratory where most of our work was done. The filter was exposed for 1 week.

3. RESULTS:

3.1.LUNG

3.1.1.DROWNING:

The results were obtained from 63 cases of drowning which had been diagnosed according to the post-mortem findings. The cases from No. 1 to No. 56 correspond to the list of cases that had also been examined for temporal bone haemorrhage, as was discussed in chapter three. There were other 7 cases which were examined for diatoms in the lung and bone marrow without being examined for haemorrhage in the middle ear. Middle ear

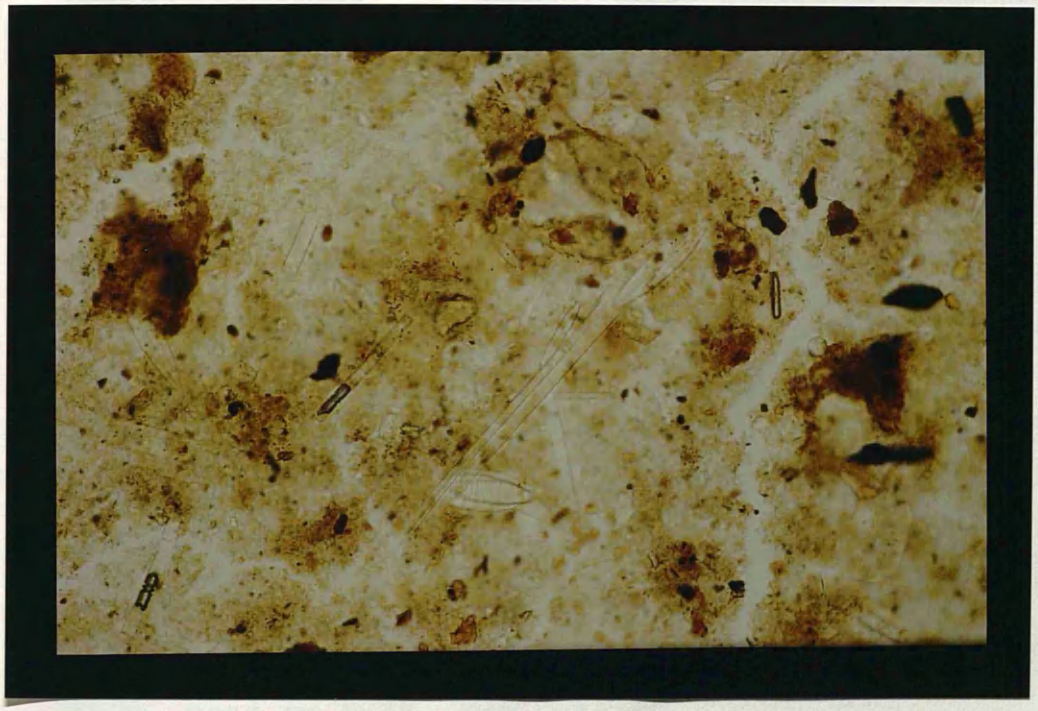

FIG.1. DIFFERENT VARIETIES OF DIATOMS FOUND IN THE LUNG OF A DROWNING CASE.

haemorrhage was examined in 29 cases of drowning without examination for diatoms in these cases. Apart from the 63 cases of typical drowning, there were 3 cases of immersion and 4 cases recovered from the water but which had been diagnosed other than that of drowning or immersion.

Table (1) shows the summary of the cases including, age, sex, cause of death, type of death, the location and time of drowning. Thirty three cases (52.3%) had drowned in the River Clyde and 6 cases in a bathtub whilst only one case had been recovered from a swimming pool. The locations in the other 26 cases of drowning were in different outdoor waters such as sea, loch, canal, pond or burn. The findings of diatoms varied from one case to another. According to the average value of diatoms in each case, taking into consideration the number of diatoms in each lobe of the lung (Table 2), no single diatom was discovered in 8 cases which represents 12% of the total number of cases. Also the average number of diatoms in 9 cases, 6 cases and 9 cases was less than one, one diatom and less than five diatoms respectively which represented 14%, 9% and 14% respectively.

This means 20 typical drowning cases which represent 31% of all the cases were either negative or contained very low counts. Most of the negative results were obtained from cases drowned in baths, a flooded burn, farm slush and the River Clyde. The average number of diatoms obtained from cases recovered from a river like

TABLE 2

SUMMMERY OF DISTRIBUTION OF DIATOMS IN THE LOBES OF THE LUNG OF BODIES RECOVERED FROM WATER . (Number of diatom/5 gm. lung)

		LEFT LUNG		RIGHT LUNG		
No.	Location	Apical	Basal	Apical	Middle	Basal
DROWNING CASES						
1	Burn	0	0	0	0	0
2	Loch	188	270	240	263	277
3	R.C.	8	12	6	16	8
4	R.C.	8	10	6	8	10
5	Swimm.Pool	0	0	2	0	1
6	R.C.	0	2	0	0	2
7	R.C.	4	0	2	0	0
8	R.C.	8	10	6	8	12
9	Bathtub	0	1	0	0	0
10	Slush	0	0	0	0	0
11	Kelvin R.	0	4	0	2	2
12	Burn	2700	4000	2450	1800	3300
13	Bathtub	0	0	0	0	0
14	Bathtub	0	0	0	0	0
15	Burn	188	222	300	196	218
16	Bathtub	0	0	1	0	0
17	R.C.	22	22	8	18	12
18	Canal	39	29	27	23	41
19	R.C.	8	22	11	12	14
20	R.C.	8	10	14	8	11
21	R.C.	55	46	51	36	48
22	R.C.	10	12	12	8	16
23	R.C.	26	33	40	22	30
24	Forth&C.	47	66	43	60	54
25	R.C.	0	4	2	6	8
26	Pond	16	14	8	18	10
27	R.C.	6	8	4	8	12
28	R.C.	0	4	2	0	0
29	Bathtub	0	0	0	0	0
30	R.C.	23	57	55	48	38
31	Loch	0	2	0	0	4
32	Sea	0	2	2	2	4
33	Sea	2	4	6	4	6
34	Sea	6	2	6	4	6

35	Loch	28	50	61	44	22
36	R.C.	23	40	44	18	22
37	Sea	0	1	0	1	0
38	R.C.	2	0	1	0	0
39	Forth&C.	2	0	0	1	2
40	R.C.	2	2	0	1	0
41	Forth&C.	6	0	2	750?	0
42	R.C.	6	16	4	6	12
43	Bathtub	0	1	0	0	0
44	R.C.	0	0	0	0	0
45	Fort&C.	1	0	0	3	1
46	R.C.	51	31	28	16	38
47	R.C.	14	10	22	6	24
48	R.C.	26	12	18	22	14
49	R.C.	2	0	4	0	1
50	R.C.	2	2	0	1	0
51	R.C.	1	0	1	0	1
52	R.C.	40	50	37	42	33
53	R.C.	37	42	40	36	52
54	River	40	38	50	44	62
55	R.C.	4	1	4	0	2
56	R.C.	28	51	27	43	16
57	R.C.	22	39	28	24	18
58	Burn	0	0	0	0	0
59	Loch	0	2	2	0	1
60	Loch	1	0	2	2	0
61	Burn	26	18	6	34	20
62	R.C.	0	1	0	2	0
63	R.C.	0	0	0	0	0

IMMERSION CASES

1A	R.C.	6	8	8	6	10
2A	Forth&C.	2	6	8	8	5
3A	Forth&C.	33	26	24	18	28

OTHER CASES

5A	R.C.	22	16	24	20	27
6A	Lake	22	14	8	4	24
7A	Ditch	38	56	40	28	61
8A	Swimm.Pool	0	0	1	0	0

the Clyde was so variable that it ranged from zero to 47 diatoms per.5 gm of lung tissue. The bodies however had been recovered from different sites of the river in different seasons. In these 30 cases of drowning, an average number of less than 10 diatoms had been counted in 15 cases and more than 10 diatoms in 13 other cases. In only 2 cases the number was 10 diatoms.

The figures obtained from cases drowned in the River Clyde showed a variation in number between one location and another during a particular time of the year, as it is shown from the cases recovered during February, May, June, August, Septmber and October. The highest numbers of diatoms that had been found in drowning cases, were 247.6, 2850 and 224.8. The first number was obtained from a case recovered from a loch, while the other two numbers belonged to cases drowned in burns.

In most cases, only a small number of diatoms were usually discovered and this required a close and careful examination by using high power magnification. The reason for this paucity of diatoms was due to a correspondingly small number of them being present in the water where the drowning took place. In these cases the diatoms are more easly missed when only 5 gm from each lobe was examined. However, this is not alawys the case as diatoms had been found in the lung of bodies drowned in water containing very few number of diatoms like cases No. 3, 4 and 25.

In 19 cases, the average number of diatoms found were

TABLE 3

COMPARISON OF NUMBER OF DIATOMS IN WATER AND LUNG SAMPLES.

CASE No.	No./100ml Water	MONTH	AVERAGE No. in lung tissue
1	0	Jan.	0
2	800	Jan.	247.6
3	10	Nov.	10
4	10	Jan.	8.4
6	25	Feb.	0.8
7	7	Feb.	1.2
8	32	Mar.	8.8
12	50.000	April	2850
15	15.000	April	224.8
17	20	May	16.4
18	33	June	31.8
19	40	June	13.4
20	22	June	10.2
21	12	June	47.2
27	10	Aug.	7.6
28	5	Aug.	1.6
30	12	Sept.	44.2
38	7	Nov.	0.6
1A	22	Mar.	7.6

TABLE 4

NUMBER OF DIATOMS FOUND IN WATER SAMPLES

Case	Location	NO.of Diatoms/100 ml.
1	Flooded burn	0
2	Sea Loch	800
3	Clyde	10
4	Clyde	10
6	clyde	25
7	Clyde	7
8	Clyde	32
12	Burn	50.000
15	Burn	15.000
17	Clyde	20
18	Canal	33
19	Clyde	40
20	Clyde	22
21	Clyde	12
27	Clyde	10
28	Clyde	5
30	Clyde	12
38	Clyde	10
1A	Clyde	22

TABLE 5

A COMPARISON BETWEEN TYPES OF DIATOMS FOUND IN WATER SAMPLES WITH THOSE DISCOVERED IN THE LUNGS OF SOME DROWNING CASES.

Case No.	Types of diatoms in lung	Types of diatoms in water
1	Nothing	Nothing
2	Cycle., Cocc.,Nav.. Synd.,Achanth.,Nitzsh. Gomphon.	Cycl.Cocc.Nav.Synd.Achanth. Nitzch.Gomphonem.Cymb.Pinn. Strait.Tabell.
3	Nav.Cocc.Himant. Bidens,Trybtion.	Nav.Nitzsh.Cyclo.Melos. Orthosira,Gomphon.
4	Nav.Pinnul.Melos.	Nav.Pleuro.Cocc.Syned.Cymb.
8	Nav.Melos.	Nav.Cocc.Melos.Gomphon.
7	Nav.Orthos.Cocc. Diatoma	Nav.Cycl.Orthos.Cocc.
8	Nitzsh.Melos.Gomphon. Cymb.Cocc.Synedra	Nav.Pinnul.Cocc.Cymb. Pleuroiz.Melos.
12	Nav.Pinnul.Eunot.Amphora Syned.Cocc.Cymb.Biddul. Grammatoph.Strat.Gomphon. Tabell.Meridion,Nitzsh. Actinopty.Pleurioz.	Nav.Melos.Orthos.Diatoma, Cocc.Cycl.Cymb.Syned.Pinnul Biddul.Grammatoph.Gomphon.
15	Nav.Cymb.Pinnul.Cocc. Diatoma,Surirel.Scizon. Gomphon.Syned.Cycl.Melos.	Nav.Grammatoph.Gomphon. Diatoma,Tabell.Pinnul. Eunot.Biddul.Trybt.Strait.
17	Nav.Schiz.Diatoma, Gomphon.Licmophora	Nav.Gomphon.Striat.Melos. Syned.Pinnul.Gomphon.

18	Nav.Cycl.Cocc.Gomphon. Achan.Fragil.	Nav.Gomph.Pinn.Striat. Tabell.Orthos.Biddulphia Diatoma,Cyclo.Cocc.
19	Nav.Gomphon.Collect. Cymb.Eunot.Orthos.Syned.	Nav.Orthos.Grammat.Melos. Cymb.Diaatoma,Tabell.Biddul
20	Nav.Gomph.Cycl.Orthos. Amphora,Tabell.	Nav.Grammatoph.Cycl.Cocc. Diatoma,Biddul.Tabell.
21	Gomphon.Nav.Syned.Cymb. Diatoma,Pinnul.Grammatoph. Tabell.Cocc.Striatella	Grammatoph.Gomphon.Nav. Orthos.Tabell.Syned.Cocc. Skelet.Coscin.Eithemia
27	Tab.Syned.Orthos.Melos. Grammatoph.Nav.Cocc. Amphora	Grammatoph.Nav.Orthos.Syned Tabell.Cocc.Epith.Rhipid.
28	Cycl.Cocc.Orthos.	Nav.Orthos.Cocc.Grammatoph.
30	Gomphon.cycl.Amphora Syned.Doryph.Melos. Tabell.Fragil.Pinnul.	Nav.Cocc.Grammatoph.Orthos Tabell.Syned.Gomphon.Eunot. Stait.Cymb.
38	Nav.Syned.Cocc.	Nav.Cymb.Syned.Cocc. Fragil.Striat.
1A*	Nav.Pinnul.Synedra	Cocc.Cymb.Nav.Melos.Synedra

* IMMERSION CASE

compared with those of their respective water samples, and this showed a relative consistency between the two sets (Tab.3). Due to a small number of diatoms present in the lung and water, it became essential to compare the types of diatoms of the two samples. Table (5) shows acceptable similarity. It should remembered that the victim may have drifted away from the actual site of drowning in many cases, and that the time which elapsed between drowning and recovery of the body is also to be taken into consideration.

The two possibilities of the place being changed and the elapsed time differential were encountered in cases No. 3 and 4, which showed a wide difference between the types of diatoms found in the lung and the water (Tab.5). Concerning the seasonal variation in number of diatoms, it was obvious that the large number had been obtained during the period between May and September as it is shown from cases recovered during that time of the year.

Concerning the distribution of the diatoms in the five lobes of the lung, 34 bodies showed a nearly equal distribution of diatoms in their lungs (Tab.2). In case No. 41 the average number of diatoms,151.6, is a highly misleading figure as it does not represent an equal distribution of diatoms in all the lobes of the lung. The number detected in each lobe were between 6 and 0 in the apical and basal lobes of the left lung respectively, and 2, 750, and 0 in apical, middle and basal lobes of the right lung respectively. In the middle lobe 750 diatoms

were found in one group under one microscopic field while the rest of filter paper was devoid of any diatoms.

3.1.2.CASES OF SUBMERSION OTHER THAN DROWNING:

The data concerning cases of submersion other than drowning, calls for the following descriptions.

1. Case No. 1A, showed some fluid in the lungs and in the stomach and was diagnosed as death due to immersion in water, together with ischaemic heart disease. In this case, the average number of diatoms was 7.6 per. 5 gm of lung tissue with a nearly equal distribution over all the lung. The detection of diatoms in the lung was consistent with the gross finding of fluid in the lung, though its quantity was negligible. The types of diatoms present in the lung seemed to be consistent with those found in the water collected from the site of drowning.

2. In case number 2A, the air passages contained a substantial amount of watery fluid mixed with mucus, but no definite froth was found. The lungs were generally congested and oedematous though not particularly hyperinflated. Since the signs of drowning were not particularly dramatic and due to presence of severe natural disease, the cause of death was certified as immersion in water, chronic bronchitis and emphysema along with acute alcohol intoxication. However diatoms were detected in the all lobes of the lungs with an average number 5.8. Although the number is small, it raised the possibility that the inhalation of water

during drowning or intrusion of fluid had taken place after death.

3. Postmortem examination of case No. 3A showed none of the typical features of drowning. However at that the time of the year, (January), the water is very cold and death would most likely have been immediate on entering the water due to vaso-vagal inhibition. However large number of diatoms were detected in the lobes of the lungs, even more than those discovered in cases of typical drownings with clear features of inhalation of water.

The three other cases of submersion had been diagnosed with causes of death other than drowning or immersion. The diagnosis was based on the lack of dramatic features of inhalation of water, as well as by the presence of natural diseases and unnatural causes which by themselves were sufficient to cause death.

4. Case No. 5A was diagnosed with fracture of the skull and inhalation of blood as the cause of death. This body was recovered from a quarry and had a large number of diatoms in the lungs with average number of 21.8.

5. Case No. 6A was a 74 years old man, who was found lying semi-conscious on the grass bank of a pond. He was taken to hospital, but was found to be dead on arrival. Necropsy showed substantial ischaemic heart disease with occlusion of one of the coronary arteries. By itself, this degree of heart disease would have been sufficient

to cause death. Ahigh level of the drug lorazepam was also detected in the blood, consistent with him having taken an over dose. There were blotchy purple patches on the forearms, knees and on the top of the feet which indicated some degree of hypothermia. The cause of death was issued as lorazepam intoxication, accidental hypothermia and ischaemic heart disease. No inhalation of water was seen in the air passages, but there was an indication of his having swallowed water, as a moderate quantity of water with a stone, the size of a box of matches were found in the stomach. The diatom test on this case showed the following number of diatoms, 22, 14, 8, 4 and 24 in apical and basal lobes of left lung and apical, middle and basal lobes of the right lung respectively.

6. The diagnosis of death in case No.7A was accidental hypothermia, acute alcohol intoxication and hypertensive heart disease, although there were doubtful internal signs such as hyperinflated lung. A striking finding, later, was the detection of many diatoms in all lobes of the lungs with an average figure of 44.6.

7. Necropsy of case No.8A showed massive pulmonary haemorrhage due to inhalation of vomit into the lung. The morbid anatomy showed abundant blood-stained froth in the trachea and bronchi, and the lungs were well expanded with massive oedema. His death was not considered to be primarily the result of drowning, although it remains a possibility that for some reason he did in fact inhale

water and partially drowned. The diatom test did not help much, as was expected from such cases of submersion where the water was almost devoid of diatoms. Only one diatom was found in the apical lobe of the right lung which was not significant.

3.2.BONE MARROW :

3.2.1.DROWNING:

The examination of the marrow was limited to that of the sternum, as it was achieved without risk of contamination by diatoms present on the skin and in the clothing of the body. Like the lung the presence of diatoms in the bone marrow depends on their presence in the water. Positive results had been found in 17 cases but with some unclear findings. Three positive cases were considered equivocal since only one diatom was detected in each case. Though the types of diatoms discovered in the marrow were similar to those of found in the lung, the 3 diatoms in bone marrow were of the same type i.e. Navicula (Tab.6). Therefore we can consider 14 cases only with significant results as far as the number and the types of diatoms are concerned.

The majority of cases, 46, failed to show any diatoms in the bone marrow. The number in the bone marrow did not seem to be related to the number in the lung as is shown in case number 2, which for example contained a large number of diatoms in the lungs (247.6), whilst only ten diatoms were found in 10 gms. of bone marrow. But the

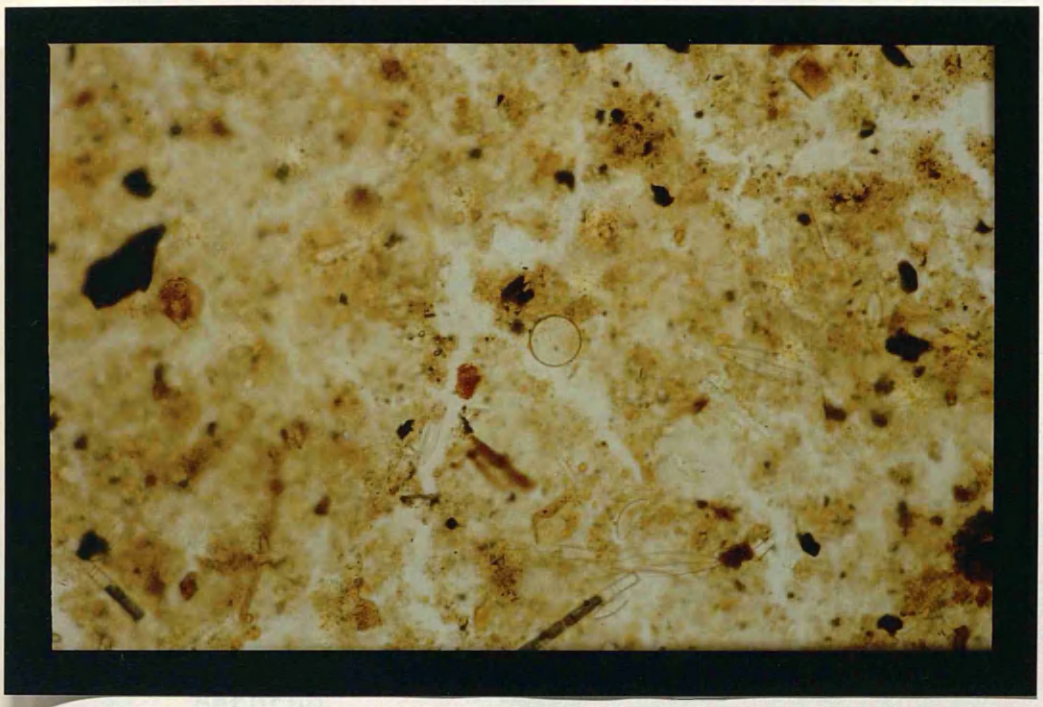

FIG.2. DIATOMS FOUND IN THE BONE MARROW OF A DROWNING CASE.

Table- 6

A Comparison between the number of diatoms found in the lung and those in bone marrow.

No.	Location	Average No. Lung(per 5gm)	Bone Marrow (per 10 gm)
1	Burn	0	0
2	Loch	247.6	10
3	R.C.	10	1
4	R.C.	8.4	1
5	Swimm.Pool	0.6	0
6	R.C.	0.8	0
7	R.C.	1.2	0
8	R.C.	8.8	0
9	Bathtub	0.2	0
10	Slush	0	0
11	Kelvin R.	1.6	0
12	Burn	2850	0
13	Bathtub	0	0
14	Bathtub	0	0
15	Burn	224.8	35
16	Bathtub	0.2	0
17	R.C.	16.4	7
18	Canal	31.8	7
19	R.C.	13.4	4
20	R.C.	10.2	50
21	R.C.	47.2	3
22	R.C.	11.6	0
23	R.C.	30.6	0
24	Forth&C.	54	13
25	R.C.	4	0
26	Pond	13.2	7
27	R.C.	1.6	0
28	R.C.	5.8	4
29	Bathtub	0	0
30	R.C.	44.2	12
31	Loch	1.6	3
32	Sea	2	0
33	Sea	4.4	0
34	Sea	4.8	1
35	Loch	41	0

No.	Location	Average No. Lung (per 5gm)	Bone Marrow (per 10 gm)
36	R.C.	29.4	0
37	Sea	0.4	0
38	R.C.	0.6	0
39	Forth&C.	1	0
40	R.C.	1	0
41	Forth&C.	151.6?	0
42	R.C.	8.8	5
43	Bathtub	0.2	0
44	R.C.	0	0
45	Fort&C.	1	0
46	R.C.	32.8	0
47	R.C.	15.2	66
48	R.C.	18.4	0
49	R.C.	1.4	0
50	R.C.	1	0
51	R.C.	0.6	0
52	R.C.	40.4	0
53	R.C.	41.4	0
54	River	46.8	0
55	R.C.	2.2	0
56	R.C.	33	0
57	R.C.	26.2	0
58	Burn	0	0
59	Loch	1	0
60	Loch	1	0
61	Burn	20.8	0
62	R.C.	0.6	0
63	R.C.	0	0
1A	R.C.	7.6	0
2A	Forth&C.	7.6	7
3A	R.C.	25.8	4
5A	R.C.	21.8	10
6A	Pond	14.4	11
7A	Ditch	44.6	0
8A	Swimm.Pool	0.2	0

most surprising finding was the failure to find any diatoms in the bone marrow, while the lung contained large numbers of them (2850 diat./5 gm) as in case No. 12. or in other cases with an average number of 20 diatoms per 5 gm of lung tissue. By contrast, cases with fewer diatoms in the lungs than those previously mentioned, showed relatively large number of diatoms in the bone marrow. These are cases No. 17, 19, 20, 27, 31, 42 and 47 with following number of diatoms in lung and bone marrow respectively; 16.4/7, 13.4/4, 10.2/50, 7.6/7, 1.6/3, 8.8/5 and 15.8/66 (Tab.6). Therefore reliance on the results obtained from the lung were hardly worth preserving in one`s endeavors for predicting the result that might be predicted in the bone marrow. The types of diatoms in the bone marrow showed some consistency with those of the water samples. In all positive cases, there are similarities between the types found in the lung and those in bone marrow, leaving no doubts about the types discovered in the three samples i.e. water, lung and bone marrow.

3.2.2.SUBMERSION CASES:

It was routine to examine all the immersion cases for diatoms in the lung and bone marrow.

In the cases of immersion the diatom test has open the door for a lot of questions to be asked about the reliability of the test in the diagnosis of drowning.

Case No. 2 and 3, for example, revealed positive findings in the bone marrow with 4 diatoms each and showing a similarity between the types discovered in the lungs and bone marrow. The other two cases of submersion, which had been diagnosed as cases other than drowning or immersion, had also shown a large number of diatoms in the bone marrow. Case No. 5 contained 10 diatoms while case No. 6 contained 11 diatoms. The types of diatoms were also similar to those found in the lungs of these cases. These findings leave no doubts that these diatoms had been transferred from lung into the bone marrow as usually happens during the process of drowning.

3.2.3. CONTROL CASES:

In order to validate the results of the diatom test from drowning cases and because of the controversial results obtained from other studies, it was decided to investigated the possibility of finding diatoms in patients who had died from causes other than drowning or immersion in water. Accordingly, 75 control cases which had been exposed to the same general environmental conditions during their lives, as those who died from drowning, were submitted to same examination.

Representative examination of the lungs showed in five cases one diatom in one lobe, two other cases showed 2 diatoms in one lobe of their lungs, while 3 diatoms were discovered in the apical lobe of the right lung in one other case. There was only one instance in which 5

diatoms had been found in the basal lobe of the left lung and one diatom in the middle lobe of the right lung.

The usual types of diatoms that were recorded, included Navicula, Grammatophora and Orthosira with an occasional finding of Tabellaria, Striatella and Nitzschia.

Bone marrow was examined in all the 75 cases using the method of incineration. Ten grams of bone marrow was used from each case, using the same technique and apparatus used with the drowning cases. Apart from one case which showed one single diatom, the other 74 cases had failed to show any diatoms in the bone marrow.

3.3.CONTROL MATERIALS, TAP WATER AND AIR:

As far as contamination is concerned, it was essential to check all the materials used in the diatom test. Hydrochloric acid, xylol, toulune, distilled water and filter papers were examined several times, as being the main sources of possible contamination. Tap water was also examined for diatoms, though it was not used in any of our work. The drying of the filter paper over a light bulb led us to suspect that the filter paper could be contaminated by air if the latter was proved to contain them. For that reason, the air inside the laboratory where most of the work was carried out, was examined by exposing the filter paper to the air for a considerable period of time.

After examination of the materials on repeated occasions, no diatoms could be discovered in any of them.

Examination of the tap water showed negative results except on two occasions, when firstly Orthosira and Grammatophora and secondly Orthosira, were discovered.

4. DISCUSSION:

The necessity in the field of forensic medicine for a definite and reliable method for the diagnosis of drowning prompted us to assess the diatom test as a means to be used for that purpose.

4.1. METHODS :

There are several techniques which have been used for the diatom test such as direct examination, chemical digestion and incineration.

The direct examination which is used on lung samples has the outstanding advantage of being reliable and simple (139), together with the fact that it shows up green algae and diatoms still appear to be alive.

The method of enzyme digestion which is used to enhance the yield of tissue-bound drugs (125), has proved to be the most successful method (181). It has the advantage of being a rapid method for the destruction of lung tissue. This technique is simple and safe, and unlike chemical digestion, large quantities of boiling acid and lung tissue are not needed. With the enzyme digestion method, we do not expect to lose any of the green algae or fragile diatoms, as happens when strong acids are used

which can only be resisted- by heavy-shelled silica diatoms. Also the technique produces results in less than one-tenth of the time taken by conventional acid degradation. Furthermore this procedure provides satisfactory information on the histological distribution of diatoms which cannot be obtained by using the chemical method.

4.2. CONTROL MATERIALS :

The diatom method has been established and confirmed as a promising tool in the diagnosis of drowning in medico-legal practice. Some maintain that it can decisively estimate the cause of death, even on decomposed cadavers as well as fresh bodies (117,168,172,175).

The reliability of the method and the influence of various factors on it have been assessed by the examination of 70 cases of drowning and possible drowning and also by the examination of 75 control cases as well as of the materials during the course of this work.

The emphasis on the examination of the aqueous solutions and other materials involved in the diatom test has come from the possibility of contamination after death (97,117,126,133). We therefore applied strict regulations to reduce the hazards of contamination by avoiding too much use of the aqueous solutions which might be contaminated by diatoms.

Repeated examination of these materials yielded no diatoms, demonstrating that our work was as free as

TABLE-7

NUMBER OF DIATOMS IN CONTROL MATERIALS, TAP WATER AND AIR.

MATERIAL	No.OF DIATOMS	TYPES OF DIATOMS
Xylol	0	
Distilled Water	0	
Hydrochloric acid	0	
Tap Water	2	Grammat.,Orthos.
	1	Orthos.
Air Filters	0	

possible from any contamination. The high percentage of negative results obtained from the control cases justified our conclusion about relative freedom of these materials from diatoms, and the tap water was found to be virtually free from any diatoms.

The diatom test can be protected from contamination by keeping the apparatus and containers clean. Tap water is to be avoided in the preparation of solutions or for cleaning purposes.

Unlike the results obtained by others (97,174), who found the tap water to be highly contaminated, our results prove that the tap water in Glasgow City is rarely found to contain more than 1-2 diatoms per. 500 ml. Tap water however was not used in any of our work, instead, distilled water was the only medium used which proved to be safe. These results confirmed the findings of other workers who denied the presence of diatoms in distilled water (97,174). So, in order to ratify these findings, we compared results obtained from lungs of control cases treated with tap water or with distilled water. The counts were similar in both occasions as no diatom could be discovered in these experiments. This justifies using Glasgow tap water or distilled water without suspicion of contamination with diatoms. Although there is no adequate direct data on the extent of air pollution by diatoms in the City of Glasgow, indirect evidence was obtained from examination of air pollution in the laboratory where most of our work was done. This

was considered as sufficient data for our work.

The results obtained by exposing filter papers to air has proved that air is nearly free from diatoms. These results are in contrast to those obtained by other workers who found large number of diatoms in the air of cities that been examined (58). The conflicting results may be merely due to regional or geographical variation. However we concluded that air pollution by diatoms is not a problem in the Glasgow area. Also, as is shown in Table (7) all reagents were found free of diatoms in 500 ml. samples of each solution. This diatom-free solution was kept for subsequent experiments. This rule was applied to all the reagents and solutions used.

4.3. SEASONAL AND GEOGRAPHICAL VARIATION OF DIATOMS :

The diatom test as evidence of drowning presupposes the presence of diatoms in the drowning medium. It is therefore essential, to check for their presence in the water beforehand, since, if there are no diatoms in this fluid, it will be futile to look for them in the body. Therefore, 19 samples of water were examined from the loci where drowning accidents had happened. The types of diatoms in these samples were compared with those of the corresponding samples recovered in the lungs.

As shown in (Tab.4), the availability of diatoms in water varied from place to place. No diatoms were found

in a flooded burn in case No. 1, while 100 diatoms were detected in 100 ml. of Hogganfield Loch water (case No. 2). Also in two other samples, 15,000 and 50,000 diatoms per. 100 ml water were found which correspond to cases No. 12 and 15 respectively. Looking at the figures obtained from the River Clyde, there was a range of 7 to 40 diatoms per. 100 ml.of water. The number of diatoms varied from one place to another, and also fluctuating throughout the year and from one time in the month to another. This conclusion was reached from examination of 16 samples of water from the River Clyde in which the idea of seasonal and geographical variation of diatoms has been confirmed as emphasised by other workers (75). It seemed, that not only the seasonal and local influences affect the number of diatoms in a particular place, but also that individual species of diatoms tend to show a degree of seasonal periodicity. There is inconsistency in the species found in both water and lung samples, as some species were seen in water but not in lung and vice versa. This is shown in cases No. 3, 4, 8, 15, and 27, that a difference in the species of diatoms is obvious. The interesting point here was the presence of the time elapse between drowning and recovery of the body. This ranged from 3 days in case No. 4 to 3 months in case No.3. Only case No.15, which showed an almost total disparity between the types in both samples, the elapsed-time was only few hours between the drowning and recovery of the body. This observation of seasonal

variation is a recognized phenomenon in certain species of diatoms (75). This phenomenon, cannot justify the importance of the comparison between the species of diatoms in drowning media and human tissue, as being an important part of the diatom test in the establishing as well as the excludion of drowning. So, such comparison will be nullified then on the ground of the that interval being elapsed from the time of immersion to the time of recovery of the body. This time was usually too long as was shown in most of the drowning cases, hence the variation in number as well as species is highly predictable. Though we compared diatoms in 100 ml. of water with those in 5 gm of lung tissue which is considered to be small amount, it would be quite informative if we knew that inhaled water is uniformly distributed throughout the lung. The drowned body is usually drafted to some distance away from the original locus of drowning and then is recovered from the other site where the diatoms pattern might be different from the original locus. For these reasons, we emphasize the importance of the presence of diatoms and their number, rather than insisting on the comparison between the species of diatoms in water and the internal organs, and so preferred using the Subtilisin-A method which required only a small piece of lung for study. This, usually 5 gm, is sufficient to represent the sample of diatoms in the whole lung. The method proved highly reliable for examination of diatoms so far as simplicity and rapidity

of a method are concerned.

4.4.CONTROL CASES :

The examination of 75 non-drowning cases revealed negative results in 67, whilst in 8, small number of diatoms were found, ranging from 1 to 5. Looking at the number of diatoms and their distribution in these positive cases, one can infer an idea about the nature and mechanism of the intrusion of diatoms into the lung which was not the result of the inhalation of water. The figures obtained from these control cases contradict the results of other workers (58,126,133,154,174), who criticised the diatom-method on grounds of the presence of them in lungs of non-drowned subjects, The positive results obtained are consistent with the results of other workers (70) who found 1-3 diatoms in 100 mg of lung tissue in two instances of 8 non-drowning cases. The negative results of the control cases, generally, are in support of other studies (172). The contradiction between the results of workers who found diatoms in the lung of non-drowned subjects and those who have not, is the very centre of credibility of the diatom method as a reliable means of diagnosing death by drowning.

It was held that the presence of diatoms every where did not discredit them as a means of diagnosis. We concluded, therefore, that the diatom content of a sample of lung due to any cause of contamination is unlikely to

vitiate that support. In addition, the bone marrow was examined in all control cases in which no diatom had been found except one which showed one Navicula whose origin could not be ascertained.

We concluded from the data reported above that,in Glasgow, diatoms are not common contaminants of air or drinking water, and accordingly the method is considered reliable in this area.

Our findings, however, contradict the results obtained by the studies of some other workers (79,126,131,133) who found diatoms in the bone marrow of non-drowned subjects. This conflicting results may be due merely to regional variation. Though most of studies had been carried out on systemic organs other than bone marrow, such as liver and kidney, the results obtained from these organs must be regarded as equivocal, as high percentage of non-drowning cases contained diatoms in their systemic organs especially the liver which is considered a filter organ for most of diatoms absorbed via the gastro-intestinal tract. Therefore, we preferred using lung and bone marrow for examination of diatoms sharing the idea of most authors who advocate these tissue as being the most suitable for the purpose (55,58,172).

The literature on the diatom method is extensive, probably because there are conflicting views on the subject, but probably also many people see diatom studies as potentially very useful and so are attempting to resolve some of the difficulties encountered. We feel we

have resolved some of the difficulties by applying very easy, simple and reliable techniques for the digestion of tissue and by strict cleaning procedures throughout the course of our work. We emphasize regular examination of control materials, though it is established for us that the main sources of contamination i.e. water and air are almost free from diatoms which gives us the opportunity to work in a particularly clean environment.

The data from controls lead us to consider the diatom test seriously as a reliable method supporting the diagnosis of drowning.

Table (8) shows the summary of published data on the presence of diatoms in non-drowned subjects, giving a clear indication that bone marrow is the most suitable and reliable tissue for diatoms assay. This is in agreement with other researchers (171,172,174,175).

4.5. DROWNING CASES

Examination of 63 cases of typical drowning, diagnosed exclusively on gross postmortem findings, the diatom test has been used as supportive evidence of inhalation of water.

The value of the diatom test has been substantiated by the results of control cases, and justifies the view that any discovery of uniformly distributed diatoms throughout the lungs is explained by the inhalation of water during the process of drowning.

In 34 cases of drowning, diatoms were found in uniform

Table-8. Summary of published data on the presence of diatoms in non-drowned subjects.

	Lung		Bone Marrow	
	+.ve	-.ve	+.ve	-.ve
Tamaska(1949)			0	7
Otto(1961)	23	5		
Muller(1963)	0	10		
Janitizki(1964)	2	6	0	8
Geissler&Gerloff (1966)	11			
Porawski(1966)	1	18	1	18
Koseki(1968)	5	26		
Schnedier(1969)	7	13		
Timperman(1969)	6	16	1	1

distribution in all five lobes of the lungs. Although the number of diatoms detected in some cases was low, it was still informative regarding the composition and distribution of diatoms. On that basis, the diatom test did actually support the diagnosis of drowning while results from 21 instances where less than 5 diatoms per. 5 gm of the lung were found, were considered as equivocal, and no definite diagnosis could be made according to the test.

The diatom test, is sometimes of no diagnostic help as shown in other 8 cases where lungs and bone marrow were devoid of any diatoms.

The presence of diatoms in the lungs is closely related to the number present in the drowning media. Since the variability of the diatom content ranged from nil to 50,000 per. 100 ml. water, it would be explained according to the seasonal and geographical variation. This might be the explanation of negative results in some cases of undoubted drowning.

Concerning the results of bone marrow, there were 15 positive cases with considerable numbers of diatoms. Three other cases showed only one diatom which was regarded as equivocal.

The number of diatoms in any organ is a matter of debate; some authors speak of hundreds, others are content with finding only a single one (117,172). However, the diatom test as supportive evidence of

drowning was valid in all positive cases so long as the distribution of diatoms in the lung and the bone marrow are taken into consideration.

In the situation where no diatoms were detected in definite cases of drowning, one may speculate that the cause of death was reflex cardiac arrest or that the amount of water inhaled was insufficient to be detected by the diatom test. However, both these explanations are dubious in our cases which showed negative diatom counts, but at the same time the inhalation of water clearly demonstrated by necropsy. In these cases the only acceptable explanation is the simple absence of these organisms from the water, as there is a period when the population of diatoms is decreased and the water may be almost devoid of them. This might explaine the absence of diatoms in some cases of drowning especially those recovered from the River Clyde, which is known to contain very few diatoms throughout the year. Obviously the negative results of bathtub and swimming pool drowning are explained on the basis of the initial finding of negative results in tap water.

Clearly there is a relationship between the volume of water aspirated and the concentration of diatoms in the water, especially when they are already scanty in the water. However, this relationship does not appear to apply to bone marrow, in which no correlation between the number of diatoms in the lung and those of bone marrow can be predicted, since some cases with a few diatoms in

the lung contained many in bone marrow and vice versa.

In some negative findings in bone marrow, it seems that the diatoms in the lungs were already so few as to be difficult to detect in a tissue like bone marrow. In other cases large number of diatoms were present in the lungs yet the sternum was almost devoid of them. The possible explanation for this contradiction is circulatory failure before the penetration of the diatoms into the circulation could occur, and death due to syncope might have supervened too rapidly. However the absence of diatoms in systemic organs does not exclude the diagnosis of drowning.

The diatom test is highly supportive evidence of submersion during life, especially if they have been demonstrated in internal organs like bone marrow.

In spite of the conflicting results, the detection of diatoms in tissue of a person presumed to have drowned is of value only if it is subjected to the following provisions; (1) that no doubts exists regarding the phytoplanktonic nature of the particles observed in the tissues, (2) that an absolute certainty exists that neither the equipment nor the reagents used in the analysis were contaminated by diatoms. This requires strict cleaning procedures which include the mandatory use of distilled water, and (3) at early time of a recovery of a body from water with the certainty that the drowning did happen originally at the site of recovery, then the similarity can be demonstrated between diatoms

found in body tissue and those in the drowning media. Furthermore the finding of the identical diatoms in bone marrow, lung and water is conclusive evidence of inhalation of water during life.

THe problem of contamination has already been discussed and reasons have been addressed for bone marrow to be selected as an ideal tissue for recovery of diatoms. The number of positive cases in this study, however, are not encouraging as only 27% of submersion cases had diatoms in their bone marrow. One of the reasons for such results is that the bone marrow receives only a small portion of the cardiac output and probably even less during drowning. Thus, few diatoms are likely to find their way into bones. A further reason for negative results may be due to examination of too small an amount of bone marrow. Because of the small number of diatoms usually found in bone marrow, it is imperative that a blank control of reagents be checked at the same time, and that precautions be taken at necropsy against contamination. The use of non-pulmonary tissue other than bone marrow does not appear to offer any advantage.

4.6. SUBMERSION CASES OTHER THAN DROWNING:

All cases which had been diagnosed as death due to immersion syndrome, had shown diatoms in their lungs. In these cases the inhalation of water is the only possible mechanism in which the diatoms could be disseminated

throughout the lungs. Furthermore in 2 of these cases diatoms were found in the bone marrow of sternum which implies that they had been absorbed into the blood stream and dispersed all over the body including the bone marrow. At necropsy, the air passages contained a substantial amount of fluid mixed with mucus, but froth was absent except in case No.3A when very little frothy fluid in trachea and bronchi was demonstrated. The lungs did not show the classical signs of inhalation of fluid, so therefore the diagnosis of immersion syndrome was based on the exclusion of other natural causes as well as the absence of classical signs of the inhalation of water. However, the finding of diatoms in these cases has created a new controversy between the diagnosis of drowning based on gross postmortem findings, and the one based exclusively on the diatom test. The most plausible explanation for this is that in cases of sudden death during immersion due to vagal inhibition there might be brief agonal respiration. The finding of a few diatoms in the lung especially in cases No. 1A and 2A, supports the belief that death from hydrocution is usually accompanied by a few convulsive gasps before dying.

The intrusion of diatoms into the lung after death is a possible mechanism due to hydrostatic pressure of water, but this can only be speculative, since the debris did not reach the periphery of the lung except in exceptional circumstances. Even if we suppose that the intrusion of diatoms into the lung post-mortem is possible, it would

be unlikely to produce disseminated uniform distribution into the most peripheral parts of the lungs. Also, intrusion of diatoms after death into systemic organs like bone marrow is virtually impossible.

Therefore, we can confidently rule out the post- mortem penetration of diatoms into lung tissue and bone marrow in bodies dying from causes other than drowning. The positive results of diatom assay do not lead automatically to a diagnosis of drowning when other conclusive evidences are considered.

The diatom test, therefore, might be negative when it is expected to be positive, and positive when it should be negative, as has happened with overt drowning cases and what are called immersion cases respectively. These findings do not invalidate the diatom test as being supportive evidence for a diagnosis of drowning.

4.7. CASES OTHER THAN DROWNING OR IMMERSION:

In 4 cases where death was due to causes other than drowning or immersion, diatoms were detected in 3 of them and in the bone marrow of 2. The post mortem findings were similar to those of immersion syndrome, but with the lacking of classical signs of inhalation of water. Severe natural and unnatural changes sufficient to cause death by themselves, were found in these cases.

Apart from case No. 8A (recovered from a swimming pool) which had only one diatom in his lung sample, large number of diatoms were found in the samples of lung and

bone marrow of other cases (No. 5A and 6A). Similar results were reported on people shot in the river Danube while trying to cross the border (151). All these died from gun-shot wounds but many diatoms were found in their organs. These results,like ours, indicate that the heart was still beating and pumping blood around the body and that some inhalation of some water had already occurred while the victim was dying from causes other than drowning. Here, again,the diatom test established inhalation and absorption of diatom-containing media into the systemic circulation. Obviously, such an absorption must have occurred in an appreciated quantity from the finding of relatively large number of diatoms in the bone marrow in 2 cases. We had the opportunity to examine a case (No.6A), found lying on the bank of a pond, to die some time later after being taken to hospital. The interesting point in this case was the finding of large number of diatoms in both lungs and bone marrow to suggest that inhalation of water had taken place, though not enough to cause drowning, since he recovered semi-conscious to die later from lorazepam intoxication, accidental hypothermia and ischaemic heart disease. Accordingly, positive results of the diatom test, sometimes, mean no more than inhalation of water rather than a definite sign of diagnosis of drowning. Drowning depends on the amount of water inhaled and the time required for that water to be sufficiently absorbed into

circulation. It is assumed, in drowning that enough fluid has been inhaled and yet the victim has survived long enough for absorption and electrolyte changes to take place. However, the diatom test is proved to be a vital phenomenon, especially if it could be positively demonstrated in internal organs like the bone marrow, indicating that life was still present before immersion in the water.

5. CONCLUSION:

The lung and bone marrow of 63 drowning cases, 7 possible drowning and 75 control cases were examined to assess the reliability of the diatom test in the diagnosis of drowning as well as the suitability of these tissue for examination for diatoms.

The risk of contamination has been assessed through examination of control materials used in the process of the method with special consideration given to air pollution.

Analysis of data from 63 drowning cases involving 16 putrefied bodies has confirmed the value of the diatom test, as a vital phenomenon. We agree with other workers (53,151) who advocate using bone marrow as the most suitable tissue. Also we recommend representative examination of lobes of lung tissue as being the best tissue for the determination of diatom compositions i.e. types, number and distribution. Although, some reported that the bone marrow from the sinus of basal bone had the

advantage of being regarded as a closed cavity (48), we found the sternum technically more convenient and equally less subject to external contamination. The use of tissue other than bone marrow and lung do not appear to have any advantage. The validity of these two types of tissue for examination of diatoms is substantiated by negative results obtained from control cases.

These results support the findings of some workers (97), and contradict the results of others (126,151) who challenge the significance of the diatom test on the basis that diatoms are common pollutants of air and water and found in the tissue of cadavers dying from causes other than drowning or immersion. In the Glasgow area, however, the finding of diatoms in the internal organs of a body recovered from water may indicate inhalation of water but does not, always, lead to a diagnosis of drowning. In situations where no diatoms were detected in overt cases of drowning a special consideration has to be paid to the seasonal and environmental variation (75). This was confirmed by the paucity of diatoms detected in cases from River Clyde between November and February. Generally, small number of diatoms had been found from cases recovered from the river where environmental conditions and water- pollution are unfavorable to their growth.

One of the most significant aspects to emerge from this study is the importance of the examination of the pattern of distribution of diatoms in lung with special regard to

their number. This has been successfully achieved by using an enzyme digestion method, used originally for drug extraction from tissue. Simplicity, facility of processing and saving of time are the advantages of this method.

The value of the diatom test is as a diagnostic tool in cases of drowning still not entirely decided, but it does confirm that absorption of diatom-containing media has taken place. The diatom test therefore cannot be considered as conclusive evidence of drowning on its own merit, but is regarded as highly supportive evidence of inhalation of water with or without drowning. The diatom test though has resolved some of the difficulties in the way of diagnosis of death by drowning, yet it cannot be considered as an ideal solution. However it is one of the remaining tools when resuscitation has been attempted or when putrefaction is advanced. There will be however the occasional doubtful results which will then have to be interpreted with the necessary due caution.

Drowning, according to the results obtained by the diatom test, is a more complicated mechanism than just the inhalation of water, so we tried to find further characteristic evidence for diagnosis and that was by the examination of the temporal bone for hemorrhage in middle ear and mastoid air cells. This will be discussed in the next chapter.

CHAPTER 3

HISTOPATHOLOGICAL EXAMINATION OF THE TEMPORAL BONE IN CASES OF ASPHYXIA, AND HAEMORRHAGE INTO THE MIDDLE EAR AND MASTOID AIR CELLS IN DROWNING VICTIMS

1. INTRODUCTION & LITERATURE REVIEW:

Attempts have been made to find a pathognomonic or characteristic sign which could be used in the diagnosis of drowning in fresh as well as putrefied bodies. To achieve this, examination of haemorrhage into the middle ear and mastoid air cells has been conducted.

The first observations at necropsy of haemorrhage into the middle ear and mastoid cavity in drowning victims was recorded in 1951 (149). After that, increasing attention had been paid to the examination of the temporal bone in cases of drowning, which had previously been ignored or seldom recorded by pathologists. Similar haemorrhage into middle ear is a recognised result of underwater swimming as a result of barotrauma (45,78).

This phenomenon has been emphasized as a characteristic feature of drowning, since it was not demonstrated in 89 cases dying from causes other than drowning (122). However, Intrapyramidal haemorrhage has been reported in other conditions such as massive intracranial

haemorrhage, fractures of temporal bone or skull, bleeding diatheses, and severe hypoxia other than drowning (136). The diagnosis of these conditions is usually straight-forward, and could be readily excluded, leaving the diagnosis of drowning as a cause of death to be considered more carefully. This phenomenon of middle ear haemorrhage in drowning has been explained on the basis of otological complications of diving.

In diving, especially in scuba divers, drowning is the leading cause of death, (186), and accounted for 60% of all causes of death in this sport. The reason is the frequent occurrence of loss of consciousness in scuba divers, leading eventually to drowning. This loss of consciousness is attributed to many causes, such as asphyxiation, arterial gas embolism, head injury, heart attack, decompression sickness and gas contamination of the tank (186). Here, much attention has been paid to tissue damage by air-containing structures following changes in the ambient pressure, and is called barotrauma. It is defined as the failure to equalize the pressure within the air-containing spaces either during descent (compression) or ascent (decompression) (45). Middle ear barotrauma occurs usually during descent which is a common hazard in diving, but it may also occur during ascent as a result of the failure or inability to equalize the pressure of expanding gas within the middle ear (45). However, in either case, the failure of equalization of pressure within the middle ear leads to

discomfort and pain with of stretching the ear drum and the eventual rupturing of blood vessels in both the tympanic membrane and the lining of the space with resultant haemorrhage and transudation into the middle ear and sinuses (99). Briefly, as the middle ear and mastoid spaces are surrounded by rigid walls. They are affected by pressure differentials which leads to the formation of a relative vacuum within the middle ear causing the inward stretching of the tympanic membrane, oedema of the lining mucosa and subsequent haemorrhage. This event will not occur if the pressure within and without the chambers are equalized (45).

Patent Eustachian tubes and sinus ostia are important safeguards and upper respiratory infections are widely understood as contraindications to scuba diving. Data obtained from experimental studies show that water may invade the tympanic cavity via the Eustachian tube (180), producing abnormal pressures which might cause haemorrhage of middle ear.

An important point derived from the pathogenesis of the lesions of the middle ears of underwater swimmers is that the haemorrhage, is not only a complication of drowning but also might be one of its causes. The mechanism of haemorrhage as a result of drowning is based on the theory that inner ear dysfunction is the final complication of the derangement in the middle ear, which may be caused by haemorrhage (122). Vertigo, caused by inner ear dysfunction, might make the swimmer lose his way under water, literally he might even not know which

way is up to the surface. This is possibly the reason for otherwise unexplained drowning in good swimmers.

An opinion formulated from observations of other conditions related to pressure distribution in the middle ear which might lead to vestibular dysfunction, e.g. severe earo-otitis (122), cassions disease (78), and skin diving (186). The mechanism is similar to that of the caloric test for equilibrium. However, in drowning, no morphological changes could be detected in the inner ear (7,102) to explain the aetiology of vertigo. However other investigators noted that the perilymphatic space of the inner ear was sometimes haemorrhagic after diving which might have been caused by air bubbles blocking the capillary circulation resulting in haemorrhage when the capillary wall begin to leak (54). Such mechanisms cannot be confirmed with certainty in drowning (93). Though some workers demonstrated the rupture of the tympanic and round window membranes in cases of scuba diving (54,177), but in drowning, neither of these membranes were found to be ruptured, though some bleeding was noticed within their layers (93).

The absence of significant morbid changes in the inner ear, a theory of anastmosis emerged to explain partly the vestibular dysfunction in drowning. The theory is based on the possibility of anastmosis between the capillaries of the middle and inner ears (180). The mechanism of vertigo has been explained by what is called Nerve theory, based on the observation that the surgical

treatment of Meniere's disease is sometimes successful in relieving vertigo by neurotomy of the middle ear (180).

Generally, barotrauma is brought about by pressure differential which operates in deep water, but such a mechanism is obviously not the sole explanation for temporal bone haemorrhage in shallow water drowning. There, the haemorrhage is explained as a result of anoxia of the middle ear, caused by the invasion of water into the upper air way. This leads to increased resorption of air and increased negative pressure and might well result in oedema of the mucosa and vascular congestion making the middle ear more sensitive to a slight amount of barotrauma (7).

Concerning the depth of water which is required to produce the lesion, it was observed that haemorrhage in middle ear with violent vertigo occurred with as little as 10 ft (99). But striking changes in the tympanum have been reported at depths as low as 2.3-4.6 ft (9). However, it is generally thought that 6 ft. may be of a sufficient level to produce these changes (9).

A third possible mechanism of intrapyramidal haemorrhage is of violent respiratory effort against the glottis locked in laryngospasm, which might result in increased venous pressure, transudation of serum into the lamina propria and the eventual rupture of the weakly supported vessels of those in the lining of these air spaces (102).

2. Material and Methods:

Temporal bones of 93 bodies recovered from water were submitted for histopathological examination. They included 85 cases of typical drowning, 4 cases of immersion syndrome and 4 bodies dying from causes other than drowning or immersion. Eighty nine right and 55 left temporal bones from cases of typical drowning were examined naked-eye and microscopically. Bilateral examination of the temporal bones was performed on the other 8 cases, except in one case of immersion syndrome where only the right temporal bone was examined.

This furnishes a total of 153 temporal bones and these were examined to determine the validity of the haemorrhage into the middle ear and mastoid air spaces as a reliable signs of death by drowning.

Among the cases of drowning there were 7 bodies discovered in bathtubs and 2 others in swimming pools, while the rest had drowned in outdoor water such as rivers, burns, the sea, sea loch, ditch or pond. Our study covered most drowning incidents within the Glasgow area, as more than half of the cases had been recovered from the River Clyde, the main waterway in Glasgow area.

Drowning was the immediate apparent cause of death in all the cases with or without other contributory disease. Acute alcohol intoxication was a contributory factor in 12 cases, while drugs such as paracetamol, butane, propanolol and temazepam were discovered in poisonous levels in 3 of the bodies. Other causes contributing to death by drowning were found in 14 cases,

TABLE-1. SUMMARY OF CASES RECOVERED FROM WATER.

Case No.	Sex	Age	Type of Death	Month	Location of Drowning
DROWNING CASES					
1	M	12	A	JAN	Burn
2	F	54	S	JAN	Loch
3	M	52	?	NOV	River Clyde
4	M	17	A	JAN	R.C.
5	M	7	A	JAN	Swimm.Pool
6	M	51	S	FEB	R.C.
7	F	64	A	FEB	R.C.
8	M	60	A	MAR	R.C.
9	F	68	S	MAR	Bathtub
10	M	39	A	APR	Slush
11	M	27	A	MAR	Kelvin R.
12	M	4	A	APR	Burn
13	F	48	S	APR	Bathtub
14	F	60	S	APR	Bathtub
15	M	33	A	APR	Burn
16	M	19	S	MAY	Bathtub
17	M	31	A	MAY	R.C.
18	M	10	A	JUN	Canal
19	M	52	A	JUN	R.C.
20	M	60	A	JUN	R.C.
21	F	13	A	JUN	R.C.
22	M	51	S	JUN	R.C.
23	M	53	S	JUN	R.C.
24	M	40	A	JUN	Forth&C.
25	M	30	A	JUN	R.C.
26	M	63	A	JUL	Pond
27	M	45	A	AUG	R.C.
28	F	45	S	AUG	R.C.
29	F	75	S	SEP	Bathtub
30	M	35-45	?	SEP	R.C.
31	F	77	S	OCT	Loch
32	M	22	A	OCT	Sea
33	M	34	A	OCT	Sea
34	M	22	A	OCT	Sea
35	M	66	S	NOV	Loch
36	F	61	?	NOV	R.C.
37	M	27	A	OCT	Sea

38	M	73	S	NOV	R.C.
39	F	71	S	DEC	Forth&C.
40	F	37	S	DEC	R.C.
41	M	70	A	MAR	Forth&C.
42	M	14	S	APR	R.C.
43	M	44	A	MAY	Bathtub
44	M	17	A	MAY	R.C.
45	M	53	A	JUN	Fort&C.
46	M	23	A	FEB	R.C.
47	F	42	S	MAY	R.C.
48	M	22	A	JUN	R.C.
49	M	28	S	JUN	R.C.
50	F	73	S	AUG	R.C.
51	F	56	A	AUG	R.C.
52	M	18	A	AUG	R.C.
53	M	52	S	SEP	R.C.
54	M	74	A	SEP	River
55	M	14	?	MAR	R.C.
56	M	?	A	FEB	R.C.
57	M	49	A	JUN	R.C.
58	M	5	A	JUL	River
59	F	40	A	JUL	River
60	M	61	A	NOV	Fort&C.
61	M	67	A	MAR	R.C.
62	M	52	A	AUG	R.C.
63	M	39	A	SEP	River
64	M	23	A	DEC	Sea
65	M	58	A	MAR	R.C.
66	M	25	A	MAR	River
67	M	24	A	JUL	R.C.
68	M	18	A	OCT	Fort&C.
69	M	42	A	JUN	R.C.
70	M	8	A	AUG	R.C.
71	M	35	A	DEC	River
72	M	62	?	DEC	River
73	M	51	A	APR	R.C.
74	F	64	?	JUL	R.C.
75	F	75	A	JUL	R.C.
76	M	5	A	AUG	Fort&C.
77	M	77	S	SEP	Bathtub
78	F	28	A	SEP	R.C.
79	F	68	A	OCT	R.C.
80	F	31	A	NOV	R.C.

81	M	5	A	JAN	Kelvin R.
82	M	59	S	APR	Burn
83	M	26	A	MAY	Forth&C.
84	M	58	A	JUN	R.C.
85	M	66	A	JUN	R.C.

IMMERSION CASES

1A	F	59	A	MAR	R.C.
2A	M	58	A	SEP	Forth&C.
3A	M	40	S	APR	Forth&C.
4A	M	7	A	DEC	Swimm.Pool

OTHER CASES

5A	M	40	A	JUN	River
6A	M	74	A	JUN	Lake
7A	M	60	A	MAR	Ditch
8A	M	26	A	APR	Swimm.Pool

TABLE-2

SUMMARY OF CONTROL CASES.

CASE No.	SEX	AGE	CAUSE OF DEATH
HANGING			
1	M	39	HANGING
2	M	44	=
3	M	64	=
4	M	23	=
5	F	54	=
6	M	50	=
7	M	18	=
8	M	39	=
9	M	63	=
10	F	54	=
11	M	70	=
12	F	21	=
13	M	48	=
14	M	38	=
15	M	23	=
16	M	20	=
17	M	55	=
CARDIAC CAUSES			
18	M	50	Herat Failure
19	M	61	I.H.D.
20	M	49	I.H.D.
21	M	45	C.A.A & T.
22	M	48	A.M.I.
23	M	58	I.H.D.
24	F	7 Days	Congenital H.D.
25	M	46	I.H.D.
26	F	70	I.H.D.
27	F	75	I.H.D.
28	M	43	I.H.D.
29	M	65	I.H.D.
30	M	42	I.H.D.
31	M	66	I.H.D.
32	M	65	I.H.D.

ASPHYXIA

33	M	65	Choking on Food
34	M	40	Inhalation of blood
35	M	10 Months	Inhalation of Gastric content
36	F	7 Weeks	=
37	M	1.5 Months	Suffoction
38	F	71	=
39	F	3	Mechanical constriction of the Neck
40	M	37	Inhlation of Blood
41	M	71	Choking by Vomitus
42	F	46	Asphyxia by Epilepsy.
43	M	43	Gastric Inhalation

INTRACRAINAL HAEMORRHAGE

44	F	60	Subarachnoid Haemo.
45	M	66	" "
46	M	26	Skull Fracture
47	F	72	Cerebral Haemorr.
48	M	77	" "

CARBON MONOXIDE POISONING

49	M	23	C0 Poisoning
50	M	21	"
51	F	34	"
52	M	18	"
53	M	25	"

MISCEALLANOUS CAUSES

54	F	2.5 Months	S.I.D.S.
55	M	53	Hepato-Renal F.
56	F	30	Fire Death
57	M	50	"
58	M	50	Bronchpnumonia
59	M	10 Months	S.I.D.S.
60	F	60	Fatty Liver
61	M	62	"
62	M	74	Hypothermia
63	M	6 Weeks	Bronhopnumonia
64	F	5 Months	S.D.I.S.

65	M	48	Chlormethiazole Intox.
66	F	66	Carcinoma of colon
67	F	54	Nitrazepam Poisoning
68	F	38	Bronhopnumonia
69	F	85	Peritonitis
70	M	53	Multiple injuries

e.g. old myocardial infarction, severe atherosclerosis of the coronary arteries, recent coronary thrombosis and fracture of the skull. The reliability of the histological examination of the temporal bone was assessed on putrefied bodies, specially those recovered from the water. Seventeen cases had been diagnosed as typical drowning, and showed advanced degree of decomposition and are included among the 89 cases of drowning.

To determine whether intrapyramidal haemorrhage is peculiar to drowning, or whether it might occur in other pathologies of death, a further group was studied. This group was divided into 5 subgroups according to the cause of death. They involved 17 cases of hanging, 15 of ischaemic heart disease, 10 of asphyxia, 5 of head injury, 5 of intracranial haemorrhage, 5 of carbon monoxide poisoning. An additional 17 cases with miscellaneous causes of death including fatty liver, bronchopneumonia, sudden infant death syndrome and drug poisoning were studied.

2.1. PROCEDURE FOR REMOVAL OF THE TEMPORAL BONE:

The petrosa, middle ear, mastoid process and external auditory canal can be removed from the skull without external mutilation if done carefully after the brain has been removed. The scalp was incised over the corona, (i.e. in the coronal plane), with a sharp scalpel, the cut passing immediately behind one external ear to that

of the other. The scalp is then released or loosened by blunt dissection from the underlying skull. Care must be taken to keep the hair and skin intact. The anterior portion of the scalp is reflected forward down over the forehead, and the posterior half is reflected backwards to the base of the skull. Using a Desoutter post-mortem saw the cranial cavity was opened by an horizontal cut on a level with the most superior part of the external ear. The brain and overlying dura mater are then removed to reveal the cranial fossae. When the brain is being removed it is advantageous to leave as much of the 7th. and 8th. nerves as possible within the internal auditory meatus. This will permit microscopical examination of the peripheral vestibular ganglion within the meatus and observation of the related structures. Before starting to remove the temporal bone, it is advisable to locate certain landmarks precisely. Starting at the incision posterior to the external ear the anterior skin flap is reflected antero-inferiorly so as to detach the external ear and locate the posterior end of the zygomatic arch, an important landmark for subsequent removal of the middle ear from the skull. The posterior skin flap is reflected backwards to reveal the mastoid process. A scalpel is then run under the mastoid process and external auditory meatus, to free the bone from the overlying structures, in particular the sternomastoid muscle. On the inner aspect of the cranial cavity the foramen ovale and jugular tubercle are identified since

they form important landmarks for the saw cuts. Block technique is recommended for removal of the temporal bone, in which three or four cuts are made by using the Desoutter saw.

1. The first cut is made near the apex of the petrous bone and anteromedial to the cochlea. The cut was passing through the foramen ovale and jugular tubercle.

2. The second cut is made roughly parallel to the first, out to the lateral wall of the mastoid region. Most of the mastoid process is necessary for histological study.

3. The third cut is made vertically down through the squomous temporal and through the floor of the middle cranial fossa on a line passing through the most posterior part of the zygomatic arch and the foramen ovale.

4. The final cut is made in a line parallel to the superior margin of the petrous temporal, just posterior to the sigmoid sinus so as to pass posterior to the mastoid process at its lateral end and through the jugular tubrcle at is medial end.

Often the second cut was omitted, and 3 cuts were enough to free the bone efficiently by using the piece of the squamous temporal bone as a handle. The block was then dissected away from the underlying structures. Once free the block was placed in 10% formalin, the cranial cavity could then be packed, and the vault of the skull replaced and the scalp reconstructed.

2.2. Fixation:

Each temporal bone was placed in a 500 ml glass jar with a sealed top, containing about 300 ml of 10% formal-saline. The jar with fixative and specimen was kept at room temperature. With a generous amount of fixative used, there is no need for the solution to be changed, but to improve penetration of the fixative, the jar may be shaken once or twice a day.

The time of fixation depends on the size of the specimens but it was usually around 7 days. For subsequent staining, this time did not seem critical, as even after a longer time formalin pigments did not appear to be in the stained section.

2.3. Sawing:

After adequate fixation the excess soft tissue was trimmed away, using a single-edged sharp razor blade. The dura mater was then removed from the superior surface of the bone and haemorrhage was looked for beneath the semi-translucent tegmen tympani.

The specimens were ready for sawing after a 7-10 days immersion in a decalcifying solution of formic acid/citrate which was used to facilitate the sawing at this stage. We used this initial period of decalcification to avoid damage to the air cells. Also, to demonstrate the haemorrhage grossly and to take some photographs, the following procedure was used:

The tegmen tympani was pierced and sufficient bone was

levered up to locate the malleus, the epitympanic recess and the promontory of the middle ear. A curved vertical saw-cut was then made, beginning in the midline of the mastoid process as a reference surface, extending medially and forward so as to pass between the malleus and the promontory as exposed by the method described above. The exposed medial surface of the middle ear and mastoid air cells were gently cleaned with cotton wool dipped in 10% formalin and was inspected for gross haemorrhage. A photograph was taken of the cut surface showing the levels of horizontal and vertical sections marked by black paper strips. The petrosa was sectioned in 3 standard classical planes. These were oriented in relation to the long axis of the petrosa, and not in the planes used in conventional anatomy. This is necessary because of the diagonal position of the petrosa in the base of the skull.

The classical planes of sections were as follow:

1. Vertically, at right angles to the long axis of the petrosa. This plane of section demonstrated 4 views of the bone.

(i) A vertical section through the apex medially to 8th nerve and through or just lateral to the 5th. nerve.

(ii) A vertical section through the bony Eustachian tube and cochlea at the level of the internal acoustic meatus.

(iii) A vertical section through the middle ear and vestibular labyrinth at the level of the mallelus.

(iv) A vertical section through the mastoid process.

2. Another vertical section at the plan of the long axis of the petrosa was taken. In order to achieve this, the simplest procedure is to place the specimen with its base i.e. infero-lateral surface, down on the mounting block. This surface should be as flat as possible by trimming and cutting away bony projections, to allow a convenient orientation of the bone on the mounting block.

The first section cut will then be through the external bony canal and continued on cutting medially with a 2-4 mm thickness. The following structures should be seen subsequently and used as landmarks for levels of cutting, the ear drum, the ossicles, middle ear cavity, the medial wall of the middle ear, and the labyrinth and the cochlea which appears as a snail shell coiled on its own axis. Next, the internal auditory meatus comes into view, showing the entrance of the 7th and 8th nerves. The petrosa according to this plane of section will be cut into 4-6 sections.

Three horizontal sections at the plane of the long axis of the petrosa were taken, as described previously. It is important to locate the malleus, the epitympanic recess and the promontory of the middle ear.

(i) The 1st section is at a level of the epitympanic recess.

(ii) The 2nd cut is at a level just above the promontory.

(iii) The 3rd cut is at a level just below the promontory.

(iv) The 4th cut is at a level of the carotid artery.

2.4. DECALCIFICATION :

2.4.1. INITIAL DECALCIFICATION:

The methods used for decalcification varied, as different methods had been used to find the most effective and efficient technique with the least injurious effect on the tissues. Some of the blocks were put in 300 ml of 10% formic acid citrate after they had been removed from the fixative solution and washed with water. This decalcifying fluid was prepared freshly at the time of use by mixing 520 gm trisodium citrate with the 1400 ml concentrated formic acid and 2600 ml water. The aim of this method was to render sawing more easier. The time needed for pre-sawing decalcification was about up to 10 days. Without this initial decalcification, it was found that the bone was difficult to saw into slices ofthe required 2-4 mm for subsequent processing and staining. The solution should be changed every 3-4 days to hasten the process.

2.4.2. E.D.T.A.: (ETHEYLENE DIAMINE TETRA-ACETIC ACID) :

After the initial decalcification and sawing, some bone sections were transferred into a slow decalcifying fluid, namely a chelating agent, Ethyelene diamine tetra-acetic acid (E.D.T.A.), prepared by mixing 5.5 gm of EDTA disodium salt, 90 ml distilled water and 10 ml formalin.

Because of the slow process of demineralization, E.D.T.A decalcification usually took 5-7 weeks. The end point was assessed manually.

There are many methods to determine the end-point of decalcification, e.g. x-ray which is not often readily available. The chemical method used at first relied on the detection of calcium in the decalcifying fluid, so that it is only when the reaction is negative, indicating that all the calcium has been removed from the tissue that decalcification can be said to be complete. The method involves the detection of calcium hydroxide or calcium oxalate. However, with experience we were soon able to determine whether decalcification was complete by the manual method. Adequately decalcified bone was soft and spongy, when squeezed between the fingers and found this palpation method a useful guide which avoided unnecessary tests being conducted, especially in the earlier stages of decalcification. We did not use the needle method to avoid damaging the mastoid air cells and other important structures.

2.4.3 FORMIC ACID CITRATE :

EDTA was found to be an efficient chelating agent without harmful effects on future staining methods of the tissue. The problem with our cases was the time factor. It was also found that tissue can be left longer than 2 weeks in formic acid/citrate without any great loss nuclear details. In our research the primary purpose was

to identify haemorrhage in the air cells. Therefore, formic acid/citrate has been used as the sole decalcifying fluid in most cases. The time needed for adequate decalcification was variable, but was usually in the range of 2-4 weeks, less being than the time needed when E.D.T.A. was used. It was not only for that reason we preferred formic acid citrate to E.D.T.A., but also because of the formation of a hard, white precipitate on the surface of the blocks, which impeded further decalcification. This had to be scraped off with a scalpel. This precipitate was found sometimes, even with frequent changes of the solution.

It was confirmed that E.D.T.A. is a very slow decalcifying fluid, and eventually we stopped using it.

2.4.4. 10% FORMIC ACID :

10% formic acid was used with some specimens. This weak acid was used with an agitator in order to speed up decalcification, in which the time was shortened to 4-7 days with satisfactory decalcification. The disadvantage of rapid decalcification however showed damaging effects on the tissue.

2.4.5. RAPID DECALCIFYING COMPOUND (R.D.C.) :

To examine the effects and reliability of other decalcifying fluids, we tried a commercial solution called Rapid decalcifying Compound (RDC), which is claimed to have the property of rapid decalcification whilst retaining excellent histological details. The

chemical constitution of this material has not been disclosed.

R.D.C. was used as the sole method in a few blocks. Also it was used as accelerator to complete the process of decalcification which failed to be reached in cases where treatment with other decalcifying fluids e.g. E.D.T.A. was unsatisfactory. Decalcification of small and thin specimens of bones with R.D.C. was certainly very rapid and complete within 24-36 hours. With larger and thicker specimens a longer period was required, usually within 36-60 hours. But, only 6-12 hours was adequate to achieve superior results after the specimens has been treated with E.D.T.A. for long periods.

Generally, we found from our experiences that 10% formic acid/citrate was the best method for decalcification of hard, dense bone like the petrosa, and even excess times over the recommended 15 days, very satisfactory. Also, subsequent staining was not adversely influenced by this method.

Briefly, we summarize the procedures used as follows:-

A)	1. Formic acid/ citrate	average 10 days
	2. Sawing into 2-4 mm blocks	
	3. E.D.T.A.	5-7 weeks
B)	1. Formic acid/ citrate	average 10 days
	2. Sawing into 2-4 mm blocks	
	3. E.D.T.A.	5-7 weeks
	4. R.D.C.	6-12 hours

C) 1. R.D.C. 24-60 hours

2. sawing into 2-4 mm blocks

D) 1. 10% formic acid 4-7 days

2. sawing into 2-4 mm blocks

E) 1. 10% formic acid citrate 3-4 weeks

2. sawing into 2-4 mm blocks

2.5. WASHING :

After decalcification it was very important for the tissue to be washed in gently running tap water for 24 hours or at least overnight. Obviously, this was to remove the excess acid, thereby facilitating subsequent staining.

2.6. PROCESSING :

Several different methods of processing were used, these are summarised as follows:-

A) Double Embedding Technique :

This method has been adopted using an automatic tissue processing-machine, the cycle being completed in 47 hours.

Two calibrated discs, 24 hours each, are required and punched out at the following intervals.

No. 1 Disc :

80% methyl spirit	2 hours
8% phenol in methylated spirit	4 hours
74% absolute alcohol	2 hours
74% absolute alcohol	2 hours
Equal parts of 74% absolute alcohol and amyl acetate	1 hour
Amyl acetate I	4 hours
Amyle acetate II	4 hours
Methyl benzoate celloidin	5 hours

No. 2 Disc :

Replace with No. 2 disc giving a further 4 hours in the same beaker of methyl benzoate /celloidin

Methyl benzoate celloidin I	4 hours
Methyl benzoate celloidin II	9 hours
Xylene	1 hour
paraffin bath I	4.5 hours
paraffin bath II	4.5 hours

B) Manual Processing :

50% alcohol	24 hours
70% alcohol	24 hours
95% alcohol	24 hours
100% alcohol	24 hours
100% alcohol	24 hours

absolute alcohol/chloroform	overnight
chloroform	24 hours
chloroform/paraffin	overnight
Paraffin (vacuum embedded)	8 hours(2changes)

C) Manual Processing :

70% methyl spirit	24 hours
Methyl spirit	24 hours
8-10% phenol in methylated spirit	24 hours
8-10% phenol in methylated spirit	24 hours
absolute alcohol I	24 hours
absolute alcohol II	24 hours
absolute alcohol/amyl acetate	24 hours
amyl acetate	24 hours
amyl acetate	24 hours
8% celloidin in methyl benzoate I	24 hours
8% celloidin in methyl benzoate II	24 hours
xylene	24 hours
xylene	24 hours
Paraffin in vacuum	12 hours
Paraffin in vacuum	12 hours
Paraffin in vacuum	24 hours

Embed in fresh paraffin.

D) 7-days machine process :

80% spirit	6 hours
80% phenol in methylated spirit	10 hours
absolute alcohol I	10 hours

absolute alcohol II	10 hours
amyl acetate/absolute alcohol	4 hours
amyl acetate I	12 hours
amyl acetate II	10 hours
celloidin in methyl benzoate	34 hours
xylene	12 hours
xylene	12 hours
paraffin	14 hours
paraffin	24 hours

2.7. VACUUM EMBEDDING :

Vacuum embedding is an essential for dense tissues like the bone. In some cases when the pump used for vacuuming was broken, the process was omitted and the difference was apparent in cutting which was much more difficult. With method (A) (process of double embedding), usually a minimum of 4 hours to 8 hours were required for satisfactory vacuuming. A similar procedure was used with method (B). In manual processing of double embedded blocks we used vacuuming for 48 hours with 3 times changes of paraffin at interval of 12 hours, 12 hours and 24 hours. While the time required in the 7-day process was 7 days.

The vacuum embedding oven consists of an air tight embedding oven attached to an exhaust pump, the degree of vacuum achieved was controlled by an attached gauge. By producing a negative pressure during impregnation, xylene and air bubbles are speedily removed from the

tissue, resulting in rapid impregnation. The technique implies transferring cleared tissues to a heated, sealed container of molten paraffin wax and applying suction to the container.

The aim of vacuuming is two folds:-

1. To remove air bubbles in the porous tissue like temporal bone.

2. To remove the clearing agent more rapidly by increasing its vaporization.

Vacuum impregnation was used in some blocks as an adjunct to normal impregnation with wax, i.e. transferring tissue to vacuum both after they have had the normal time of wax impregnation in the oven at atmospheric pressure.

2.8. EMBEDDING :

The paraffin used was found to be better if it was one of the plastic waxes. We used a blend of paraffin and a plastic polymers called 'Polywax' with a melting point 57C°. This type of wax is especially recommended to facilitate sectioning of tissues of varying consistency. The required hardness of the wax in order to give the necessary support for the bone to be cut in thin sections has been achieved by using the polywax. However, if the block had been cut too thin before processing, it became a problem as the block tended to "come out" of the paraffin. Therefore, it was advisable to provide the blocks with a thickness of about 4 mm to overcome this difficulty. Also, when "blocking" it was important to

have the block as flat as possible in order that when "paring in" a "full face" for the section could be obtained.

We encountered difficulty in embedding, relating to the temperature of the paraffin used, on the hot plate. If it was too hot i.e. above 56 C^{o} the bone became distorted and buckled making it liable to fall out of the paraffin. Therefore, it was critical that the temperature at the time of blocking should be the same in the oven paraffin path and the hot plate where the blocking is performed

2.9. SECTIONING :

Surface decalcification of the block was usually required to get a full section after paring in, as the presence of the mineral deposit in the tissue block was usually the case in most of drowning specimens, which subsequently prevent satisfactory sectioning.

A neutral decalcifier was used in some cases. It is made from a saturated solution of citric acid which is diluted with water 1/10 and then strong ammonia (100-160 ml) being added until PH of 7 is reached. The Saturation point for citric acid is 88.6 gm/100 mls.of water. Sometimes, 5 ml of neutral decalcifier with 2 drops of R.D.C. was used in order to get surface decalcification but, it was found that the tissue would swell up in the wax and this eventually created further difficulties.

Von' Ebners Solution was used as a surface decalcifier for half an hour, and this gave satisfactory results. It

consists of a saturated solution of sodium chloride with a drop of concentrated hydrochloric acid.

2.9.1. MICROTOMES AND KNIVES :

We were able to use a rotary microtome for cutting the bone blocks. This being achieved by using small blocks which had been decalcified satisfactoryly. Each temporal bone had been cut into 5-6 sections of 2-4 mm thickness. The cut being either in the vertical or horizontal plane, according to the type and level of section desired. Each block was divided into 3-4 smaller blocks, since each small block contained the structure needed for examination such as middle ear, cochlea, mastoid area etc. By using this technique we achieved satisfactory histological sections with a"LKB" rotary microtome.

Disposable blades are well-sharpened (160 mm x 44 mm x 12 mm) plain wedge knife. The thickness of the sections obtained were between 6-8 μ.

We found cutting was made easier if the blocks had been cooled for a while. Therefore we used plastic bags containing ice and sufficient water to form a flat tablet of ice when frozen.

Large sections which could not be accommodated in the ordinary tissue cassettes were cut by a Jung-K motorised microtome which is a very rigid, stable microtome with a slow steady cutting action. It is basically a heavy duty sledge microtome with the object slide moving in dove-tailed groves against a fixed knife. The knife used is

stout and heavy, and it measured 85 mm x 120 mm x 11 mm.

2.9.2. FROZEN SECTIONS :

Frozen sections of the middle ear and mastoid area were used with a newly manufactured cryostat called the Bright Starlet Cryostat. Sectioning was successful with both undecalcified and decalcified blocks, which were frozen to -20 C° and cut at a thickness of 10 μ. The technique created no problems, but trying to getting the sections to adhere to the slides was difficult and often impossible. For that reason, chrome alum gelatinized slides were used, but whenever staining was proceeded the sections floated off. However, the technique as a whole was abandoned. If more time had been available, the technique could have been developed, as the freezing of the bone made it simple to cut.

Regarding sectioning of the blocks, only one case was impossible to cut by normal methods, Case No 56. It is presumed that since the body had been in the Irish Sea, sand had some how impregnated the middle ear and mastoid air cells, this made cutting completely impossible. Therefore the "Sello-tape" method was tried.

2.10. STAINING :

All sections were routinely stained with haemtoxylin and eosin stains. The nuclear stain was intensified by longer exposure especially in cases following excessive decalcification. The normal time for routine tissue staining was found to yield too pale results. The

procedure was as follows:-

1. Take section to water through;

   i) xylene (Dewax the block) 5 minutes

   ii) Alcohol 2 minutes

   iii) Methylated spirit 3 minutes

   iv) wash in water 2 minutes

2.Stain with Harris's or Gill's haematoxylin, 5-7 minutes

3. Rinse off excess stain with water.

4. Slight differentiation in 1% acid alcohol

(90 ml of 95% methylated spirit + 5ml of 1% HCL)

(5-10 seconds).

5. Wash well in water.

6. Blue in Scott's tap water substitutes (S.T.W.S.)

7. Wash well in water.

8. Stain with eosin (time depends on type of dye)

9. Rinse in water

10.Take up to xylene through; ascending strengths of methylated spirit.

   i) dehydrate in methylated pure spirit

   ii) dehydrate in absolute alcohol

   iii) clear in xylene

11. Mounting.

Other staining schedules than H. & E. were used giving satisfactory results in most of the cases except in some where the stainability of the tissue was poor. Initially, we blamed the staining as being inefficient. but as long as all these cases had been treated with the same

decalcifying fluid (10% formic acid) for 4-7 days, hence the injurious affect on the tissue, so far as staining is concerned, was attributed to the decalcification method rather than the staining technique.

The schedule used for the 2nd method was:

1. Take to water through;
   - i) Dewax in xylene
   - ii) alcohol
   - iii) Methylated spirit
   - iv) Wash in water
2. Lugol's Iodine
3. Rinse in water
4. 5% 'hypo' (sodium thiosulphate)
6. Stain with Harris's haemtoxylin 2 minutes
7. Rinse in water
8. Differentiate in 1% acid/alcohol 5-10 seconds
9. Rinse in water
10. Blue in S.T.W.S.
11. Wash in water
12. Stain in 1.6% eosin in 0.7% calcium chloride, 30 minutes.
13. "D.C.M." (Dehydrate - alcohol, clear in xylene, mount).

The first schedule of H. & E. has been used as a routine in almost all the cases. The stainibility of the tissue including the mucosa of the air spaces and red blood cells was satisfactory.

Our aim was to recognise haemorrhage, by demonstrating

the red blood cells within the middle ear and air cell cavities or their mucosa. However, some cases with an advanced degree of decomposition have shown histologically some degree of autolytic changes of the mucosa or unrecognized post-mortem artefact.

Submucosal and focal haemorrhage had to be differentiated from autolytic lamina propria. Such differentiation was difficult with H. & E. stains. Therefore, special stains were used in these cases. However, it became a routine to use these stains on selected sections of all the cases. These special methods of staining were found to help in some instances for re-checking the severity of haemorrhage after being examined with H. & E. stains.

These methods included:-

A. Modification of Mallory's Trichrome Method.

1. Section to water

a. dewax in xylene 5 min.

b. alcohol 2 min.

c. methylated spirit 3 min.

d. wash in water

2. Stain with celestin blue

3. rinse in water

4. stain with haemtoxylin

5. rinse in water

6. blue in S.T.W.S.

7. Saturated aqueous picric acid 2 min.

8. Wash in water till only the red blood cells remain yellow.

9. Stain with orange G./ picric. 2 min.

10. wash in water till only the red blood cells remain orange-yellow.

11. Ponceau fuchsin (poncea 2 R). 3-5 min.

12. Rinse in water.

13. Differentiate in weak differentiator 1-2 min.

14. Rinse with water.

15.Stain with 1% aqueous light green 1 min.

16. Rinse in water.

17. D.C.M.

B. The Standard M.S.B.(Martius,Scarlet Red,Blue):

The technique employs;

Martius Yellow (Acid Yellow 24)

Brilliant Crystal Scarlet (Acid Red 44)

Soluble Blue (Methyl Blue)

Procedures:

1. Section to water (as explained before)

2. Rinse in alcohol

3. Stain with Martius Yellow. 2 min.

4. Rinse in water.

5. Stain with Brilliant Crystal Scarlet.10 min.

6. Rinse in water.

7. Treated with phosphotungstic Acid.10 min.

8. Rinse in water.

9. Stain with Soluble blue. 10 min.

10. Rinse in water.

11. D.C.M.

3. RESULTS :

One hundered and sixty two cases (162) were arranged under the headings of typical drowning, death from immersion in water, death due to other causes than drowning, hanging, asphyxia, carbon monoxide poisoning, head injury, ischaemic heart disease and miscellaneous causes of death including fire death, sudden infant death syndrome, nitrazepam poisoning, chlormethiazole poisoning, peritonitis, bronchpnumonia, multiple injuries and sudden death in fatty liver cases.

The results have been described according to the naked-eye as well as microscopical examination of the temporal bone. The outer surface and dorsal section of temporal bone was examined in every case. As the skull was open and the brain removed, the dura matter was stripped from the petrous bone and any discoloration of middle ear was seen beneath the semi-translucent tegmen tympani.

The macroscopical examination of the outer surface of the petrous bone is considered a necessary part of the examination and was often helpful in assessing the degree of haemorrhage but it was not sufficient to be conclusive. However, all the bones from patients recovered from water were submitted to further hiostological examination irrespective of any discoloration of the outer surface of the bone. The same rule was applied on the other groups of cases, though we examined specimens presenting with abnormal discoloration of the bone.

TABLE- 3

SUMMARY OF MACROSCOPICAL, MICROSCOPICAL AND DORSAL SECTION EXAMINATION OF RIGHT AND LEFT TEMPORAL BONES, RESPECTIVELY, IN VICTIMS RECOVERED FROM WATER, REGARDING THE SEVERITY OF EACH CATEGORY AND PERCENTAGE OF AIR CELLS AFFECTED BY I.C.H.

CASE No.	% OF I.C.H.	MACROSCOPIC. APPEARANCE	MICROSCOPIC. APPEARANCE	DORSAL SECTION EXAMINATION
1	8%	+++	+	+++
2	74%	+	+++	+++
	62%	+	+++	+++
3	94%	++	++	+++
4	95%	+++	+++	+++
5	35%	+	+	(Dark pinkish discoloration
	50%	+	+	with haemorrhagic spots)
6	75%	+	+	Congestion with few haemrr.
	77%	+	+	spots
7	30%	+	+	Few haemorr. spots
	35%	+	+	
8	33%	+	+	Dark-brownish discoloration of
	36%	+	+	middle ear & mastoid area.
9	5%	+	+	Brownish spots of middle ear
	19%	+	+	& mastoid areas.
10	20%	++	+++	+++
	54%	++	+++	+++
11	95%	++	+++	+++
	97%	++	+++	+++

CASE No.	% OF I.C.H.	MACROSCOPIC. APPEARANCE	MICROSCOPIC. APPEARANCE	DORSAL SECTION EXAMINATION
12	31%	+	+++	Few dark brownish spots
	48%	+	+++	of mastoid area.
13	-	-	-	(R.) nothing
	76%	+	++	(L.) Few haemorr. spots
14	17%	+	+	(R) Dark brownish spots
	-	-	-	(L) Nothing
15	51%	+	++	haemorr. spots of middle
	53%	+	++	ear & mastoid area.
16	47%	++	+	+++
	41%	++	+	+++
17	100%	+++	+++	+++
	80%	+++	+++	+++
18	36%	-	+	Dark-brownish discoloration
	30%	-	+	of middle ear& mastoid areas.
19	90%	+++	+++	+++
	65%	+++	++	+++
20	60%	-	+	Dark-brownish discol. of tympano-
	48%	-	+	mastoid area.
21	82%	+++	++	Dark-brownish discol. of
	71%	++	++	tympano-mastoid area.
22	62%	+	+	Haemorrhagic spots of tympano-
	69%	+	+	mastoid area.
23	75%	+++	++	+++
	92%	+++	++	+++
24	75%	++	+	+++
	90%	++	+	+++

CASE No.	% OF I.C.H.	MACROSCOPIC. APPEARANCE	MICROSCOPIC. APPEARANCE	DORSAL SECTION EXAMINATION
25	84%	+++	+++	+++
	90%	+++	+++	+++
26	83%	+++	+++	Haemorrhagic spots of
	90%	+++	+++	tympanomastoid area
27	54%	+	++	Haemorrahgic spots of
	55%	+	++	middle ear and mastoid
28	57%	++	++	+++
	100%	++	++	+++
29	0	-	-	Few faint brownish spots
	12%	++	+	of middle ear and mastoid.
30	78%	++	+	Haemorrahgic spots specially
	35%	++	+	in the (R.) side.
31	34%	++	+	Haemorrh. spots
	51%	++	+	" "
32	54%	+++	+	Dark-brownish spots of tympano-
	49%	+++	+	mastoid area.
33	64%	++	++	dark-brownish discoloration of
	93%	+	+	the tympano-mastoid area.
34	78%	+	+	Dark-brownish discol. of the
	82%	+	+	tympanomastoid area.
35	47%	++	+	Dark-brownish discol. of tympano-
	91%	++	+	mastoid area.
36	95%	+	++	+++
	86%	+	++	+++
37	?	+	?	Haemorrahgic discolortion
	?	+	?	" "

CASE No.	% OF I.C.H.	MACROSCOPIC. APPEARANCE	MICROSCOPIC. APPEARANCE	DORSAL SECTION EXAMINATION
38	86%	-	+	Haemorrahgic spots of
	80%	-	+	tympano-mastoid area.
39	66%	+	+	Haemorragic spots
	62%	+	+	" "
40	92%	+++	+++	+++
	93%	+++	+++	+++
41	45%	+	+	Haemorrahgic spots
	47%	+	+	" "
42	90%	+++	+++	+++
	94%	+++	+++	+++
43	3%	-	+	Nil
	2%	-	+	Nil
44	63%	++	+	+++
	76%	++	+	+++
45	83%	++	+	+++
	85%	++	+	+++
46	84%	+++	++	+++
	91%	+++	++	+++
47	69%	+	++	+++
	65%	+	++	+++
48	92%	+	+++	+++
	89%	+	+++	+++
49	86%	++	+++	+++
	89%	+	+++	+++
50	53%	+	+++	+++
	59%	+	+++	+++

CASE No.	% OF I.C.H.	MACROSCOPIC. APPEARANCE	MICROSCOPIC. APPEARANCE	DORSAL SECTION EXAMINATION
51	92%	++	+++	+++
	91%	++	+++	+++
52	81%	+	+++	+++
	78%	+	+++	+++
53	51%	+	+++	Dark-brownish spots
	64%	+	+++	" " "
54	83%	+	++	+++
	69%	+	++	+++
55	84%	++	++	+++
	84%	++	++	+++
56	92%	+++	+++	+++
	100%	+++	+++	+++
57	57%	?	++	?
58	100%	?	++	?
59	33%	?	++	?
60	66%	?	++	?
61	50%	?	+	?
62	80%	?	+	?
63	-	-	-	-
64	67%	?	+++	?
65	88%	?	+++	?
66	90%	?	+++	?

CASE No.	% OF I.C.H.	MACROSCOPIC. APPEARANCE	MICROSCOPIC. APPEARANCE	DORSAL SECTION EXAMINATION
67	76%	?	++	?
68	73%	?	+++	?
69	80%	?	+++	?
70	60%	?	+++	?
71	90%	?	+++	?
72	13%	?	+	?
73	60%	?	+++	?
74	40%	?	++	?
75	55%	?	++	?
76	52%	?	++	?
77	66%	?	+++	?
78	67%	?	+++	?
79	66%	?	+++	?
80	-	?	-	?
81	15%	?	++	?
82	85%	?	+	?
83	0	?	-	?
84	84%	?	+++	?
85	76%	?	++	?

The petrous pyramid, both on its anterior and posterior surfaces and mastoid areas of the temporal bone were the usual sites for haemorrhage to be looked for. The importance of learning the nature of the dark reddish discoloration of the bone, was the reason to examine it, in addition for clarification of its cause. Furthermore, dorsal section examination of the bone was carried out to assess the severity of middle ear and mastoid air cell haemorrhage. It is an essential part of the examination of the temporal bone.

For outer surface examination an arbitrary standard for the degrees of severity was set up in order to categorize the main features of intrapyramidal haemorrhage. Table (3) shows the description of severity of this outer surface, dorsal section and microscopy of temporal bone haemorrhage in cases of drowning.

Microscopically, we concentrated on three important types of haemorrhage in the tympano-mastoid areas, as well as for any morbid changes of the external, middle and inner ears.

The 3 main types of haemorrhage, which were considered in our study were intracavitary, submucosal and focal haemorrhages. The first type of haemorrhage i.e. intracavitary haemorrhage means finding free blood inside the air cell cavity, either in its fresh state as recognizable red blood corpuscles, or in an haemolysed condition as in putrefied bodies. This type of haemorrhage has been classified into 3 different degrees of severity.

The first degree is mild and refers to finding a thin layer of blood lining the air cell mucosa or a few erythrocytes extravasated into the air cell cavity. In the moderate degree, one third of the air cell cavity is covered with blood or copious blood is seen extravasated into the air space. In the third degree, the haemorrhage should involves more than one third of the air space to be considered severe.

The submucosal haemorrhage, which involves bleeding within the lining mucosa of the air cells, is not confined to the area around engorged blood vessels only, but spreads to involve the adjacent mucosa. It is also divided into mild, moderate, and severe. Mild haemorrhage involves one defined area of endosteum showing haemorrhage, and differs from focal haemorrhage by involving a wider area. In moderate haemorrhage, two defined, separate areas of mucoperiosteum are involved or a continuous area less than one third of the mucosa, shows haemorrhage. In severe degree, three defined areas show the bleeding, or more than one third of the mucosa is involved in one continuous patch.

Focal haemorrhage or petechial haemorrhage is defined as extravastion of red blood corpuscles around engorged or ruptured blood vessels. Such haemorrhage keeps close vicinity around the blood vessel and does not spread far.

Mild, moderate and severe degrees are correlated to the finding of focal haemorrhage with one, two or more of blood vessels respectively in each air cell.

The degree of haemorrhage is based on a qualitative assessment of the amount of haemorrhage in every air cell, a general idea about the severity being obtained from the majority of air cells involved with a particular haemorrhage.

For grading the haemorrhage according to the number of air cells involved with each type of haemorrhage, three grades have been applied as I, II and III. Grade I means involvement of 1% to 20% of air cells with any particular haemorrhage, Grade II comprises more than 20% but less than 50%. In Grade III, the haemorrhage involves more than 50% of the air cells.

3.1. TYPICAL DROWNING :

All typical drowning cases showed varying degrees of discoloration of the outer surface of the petrous bone.

The colour was usually dark-brown to dark reddish brown, which were classified into slight, mild, moderate and severe according to the intensity of the discoloration and the area of temporal bone involved.

The depth of discoloration was identical in both temporal bones in almost all cases, except for a few which showed a slight difference.

Initially, it was thought that the intensity of discoloration might reflect the actual severity of the intrapyramidal haemorrhage. This view was disproved by some results obtained from the examination of the dorsal sections and microscopy. All cases of typical drowning

showed some degree of discoloration of the middle ear area externally ranging from mild, moderate or severe. This reflected the severity of haemorrhage internally, certainly not in all cases, but at least in a reasonable number of them (Tab.3). However, it could not be taken as a perfect indicator of intrapyramidal haemorrhage in all cases of drownings, but it was more reliable in cases which showed moderate to severe external discoloration of the bone.

Dorsal section examination of the bone appears to be valuable approach in demonstrating the intrapyramidal haemorrhage (Figs.1,2)

There were 4 cases with variable grades of haemorrhage, even though they failed to show any abnormal appearance externally. Case No 43 is explained as being involved with very mild grade as well as degree of haemorrhage, but no justified explanation for Cases No 18, 20 and 38 could be found.

Two right temporal bones and 1 left showed no discoloration of the middle ear and mastoid area. Here the explanation for these negative results was bad pneumatization of these bones as subsequently shown microscopically.

Usually 5-7 histological sections were examined from each temporal bone. The aim was to look for haemorrhage in the mucosa as well as the spaces of the air cells i.e. intracavitary haemorrhage which does not occur without a good degree of pneumatization. We concentrated on fully penumatized cells. It proved difficult to recognise

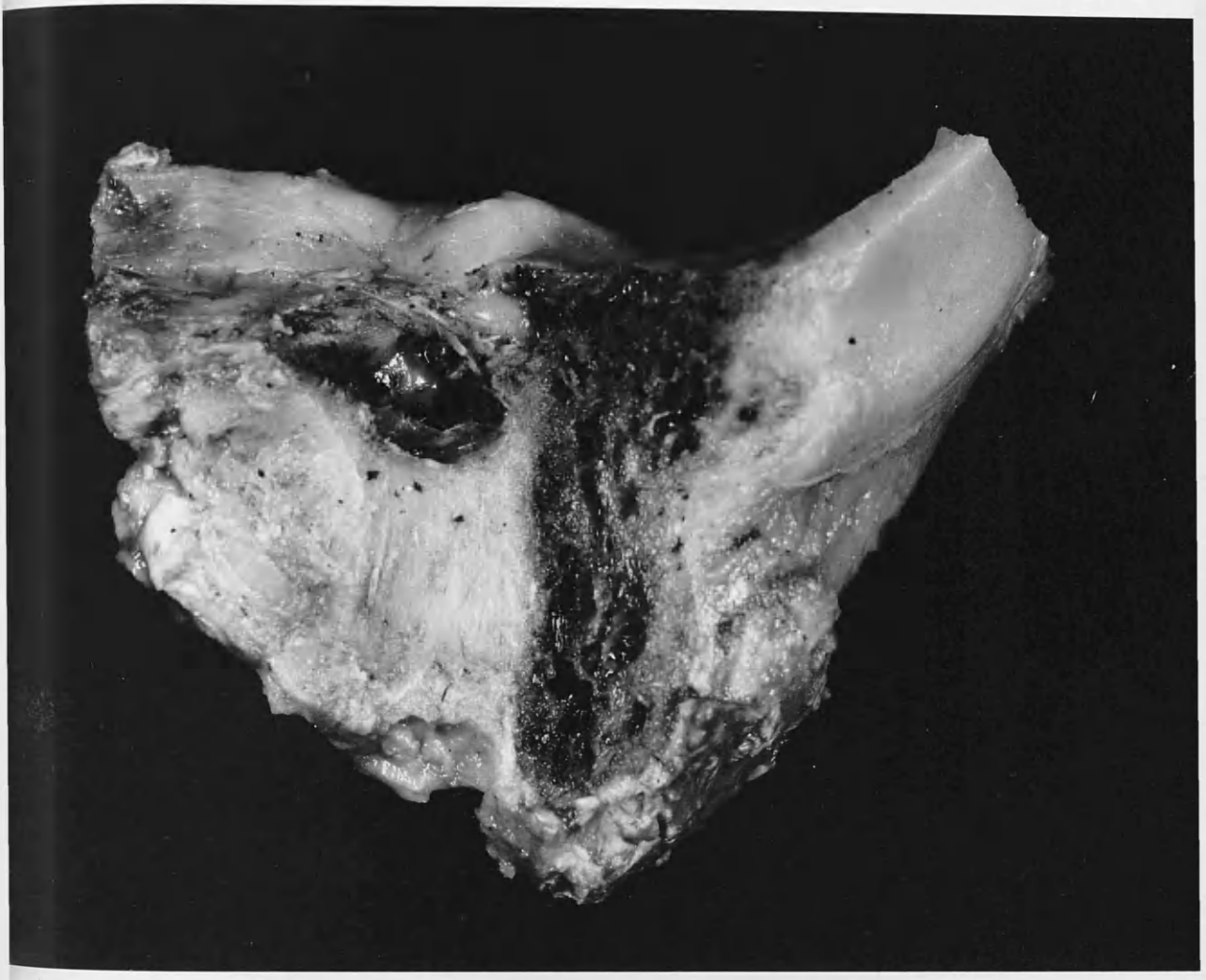

Fig. 1. Dorsal section of the temporal bone, from a drowning case, showing severe haemorrhage of the middle ear and mastoid air cells.

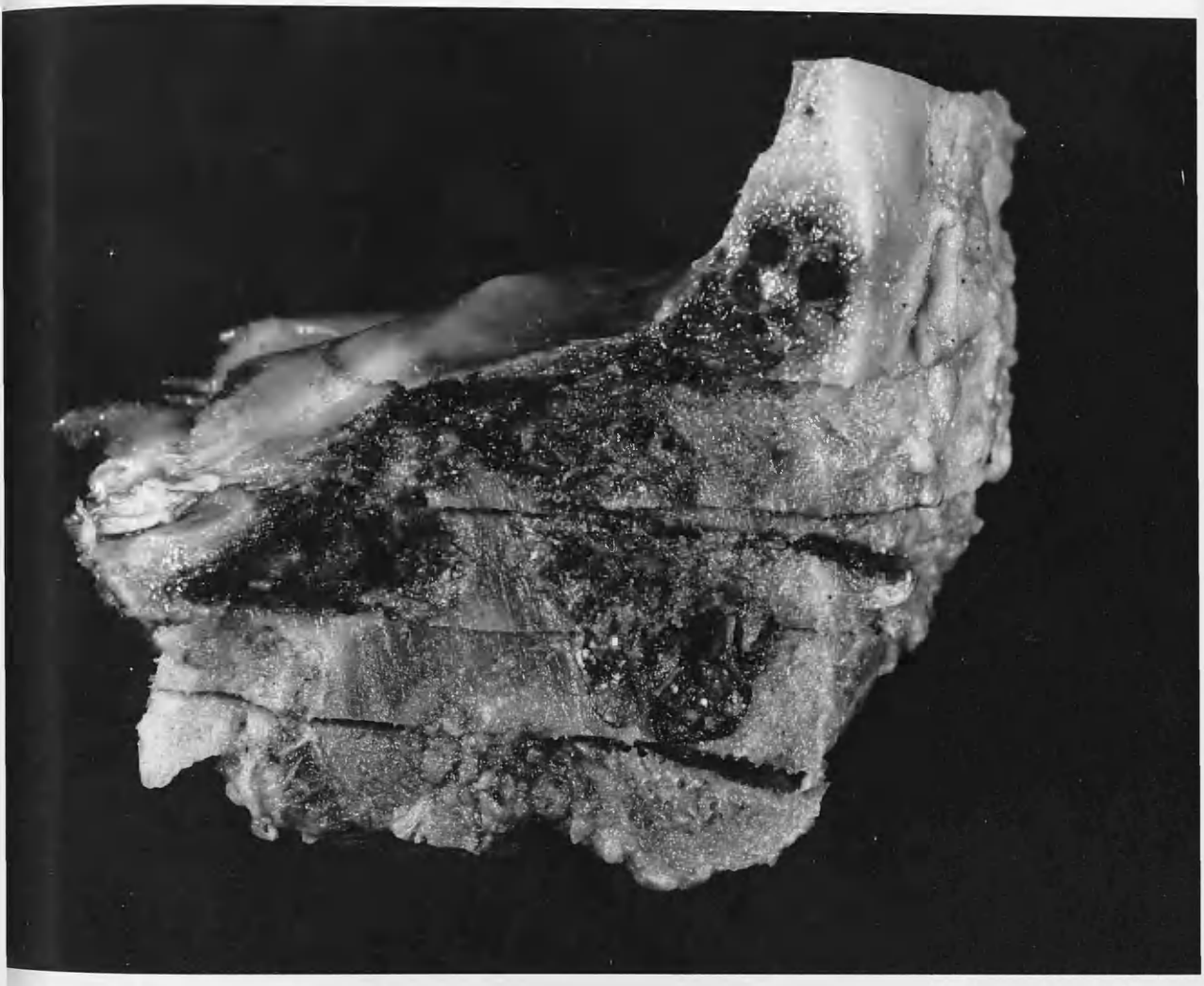

Fig. 2. The levels of horizontal sections are shown in haemorrhagic temporal bone of a drowning case.

changes in poorly penumatized air cells apart from scattered foci of focal haemorrhage around engorged vessels which were found particularly with partially diploic and partially penumatized air cells. Fortunately most temporal bones were well penumatized on both sides. Both sides of temporal bones were usually examined to identify any difference between the left and right sides.

All the air cells were counted under low power magnification and checked with medium power magnification.

The normal air cells showed no abnormal features, (including haemorrhage), but sometimes showed congestion of blood vessels or oedema of the lamina propria.

The number of bones examined, were 85 right and 55 left from 85 cases of typical drowning; among them 55 cases whose right and left bones were included in this study. The summary of the cases is shown in Table (1).

3.1.1. INTRACAVITARY HAEMORRHAGE:

Around 75% of the cases showed intracavitary haemorrhage (I.C.H.) in over 50% of the cells i.e. more than half the number of well penumatized cells (Table 4).

The number of cases exhibiting each grade of haemorrhage is summarised in Table (9-11), which showed 75% of right side and 73% of left side temporal bones with Grade III type of I.C.H., this being readily recognizable (Figs.3,4 and 5), even in cases in advanced

stages of autolysis.

Sixty one right bones out of 81, and 38 left bones out of 54 showed blood in over 50% of the air cells (Tabs.18 and 19). The degree of haemorrhage in the right temporal bones, was severe in 25 specimens, while 24 and 30 specimens showed moderate and mild degrees respectively (Tab.12). On the left side 20, 17 and 15 of the specimens were affected by mild, moderate and severe degrees of haemorrhage respectively.

As shown in (Tab.12), 2 right and 1 left bones failed to demonstrate any haemorrhage at all. These specimens belonged to cases of drowning in swimming pools and bathtubs. In other 4 specimens of the right bone, and one specimen from the left, bad pneumatization or difficulty in sectioning was the reason to put them aside without deciding whether or not they were negative (Tab.8).

From 55 cases examined bilaterally, 51 of them have confirmed I.C.H. on both sides. (Tab.7); 3 cases were positive on only one side (Tab.8). One case in which both temporal bones failed to be sectioned, has been excluded from the results. Although severe intracavitary haemorrhage has been described in more than one third of air cell cavities, most of the severe cases of typical drowning contained large amounts of blood which sometimes filled the entire space of the cell. The distribution of haemorrhage of any degree was not uniform and consistent throughout the tympano-mastoid space. It varied from one air cell to another. It may be of severe degree in some

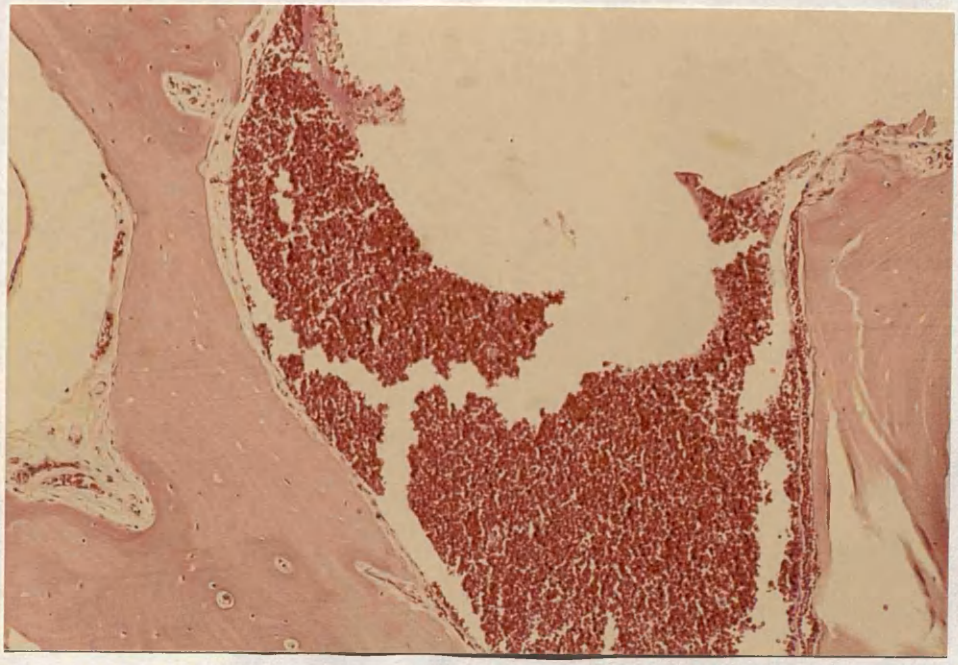

Fig. 3. Intracavitary haemorrhage of mastoid air cell.

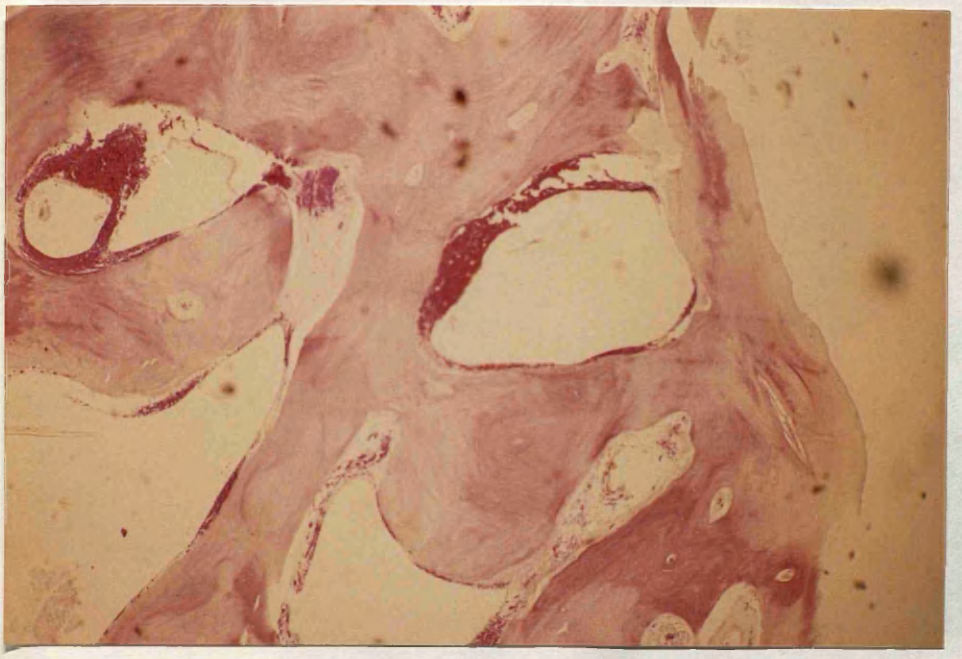

Fig. 4. Submucosal haemorrhage of mastoid air cell.

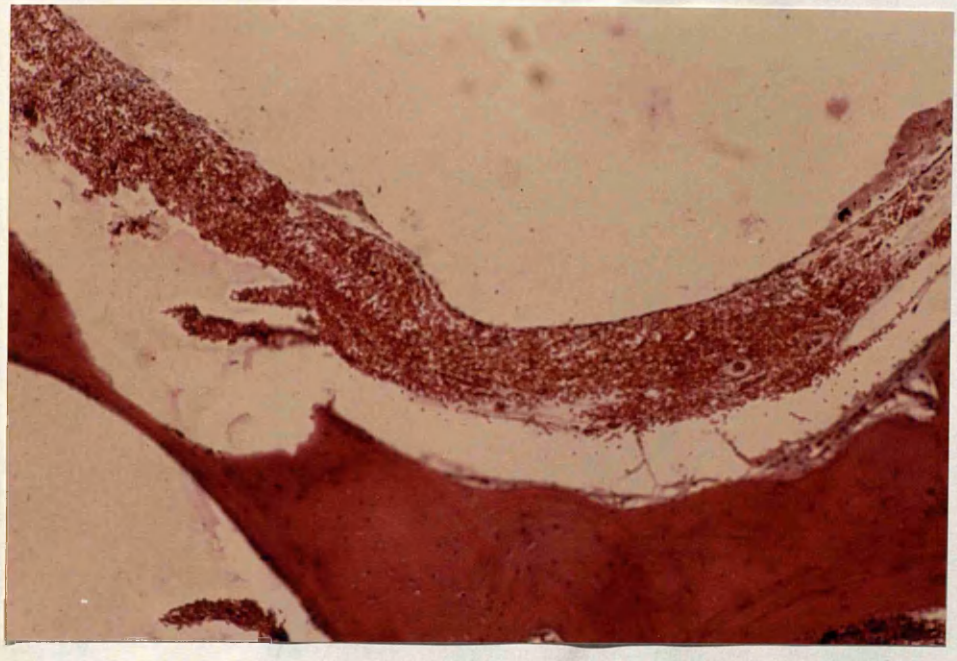

Fig. 5. Submucosal haemorrhage of the middle ear mucosa.

TABLE-4

SUMMARY OF PERCENTAGE OF NORMAL AIR CELLS AND THOSE AFFECTED BY INTRACAVITARY, SUBMUCOSAL AND FOCAL HAEMORRHAGE, OF THE RIGHT TEMPORAL BONES OF TYPICAL DROWNING CASES.

No.	NORMAL%	I.C.H.%	S.M.H.%	F.H.%
1	0	8%	75%	20%
2	8%	74%	6%	29%
3	0	94%	?	?
4	0	95%	94%	9%
5	9%	35%	54%	35%
6	12%	75%	0	12%
7	15%	30%	46%	7%
8	41%	33%	25%	33%
9	53%	75%	63%	13%
10	7%	20%	61%	26%
11	0	95%	?	?
12	34%	31%	37%	21%
13	64%	17%	7%	17%
14	48%	51%	9%	16%
15	52%	64%	?	?
16	0	100%	?	?
17	7%	36%	55%	36%
18	9%	90%	4%	20%
19	6%	60%	?	?
20	0	82%	52%	47%
21	37%	62%	0	0
22	15%	75%	62%	30%
23	12%	75%	?	?
24	0	84%	?	?
25	10%	83%	?	?
26	30%	54%	51%	18%
27	42%	57%	44%	32%
28	80%	0	0	20%
29	21%	78%	27%	6%

NO.	NORMAL%	I.C.H.%	S.M.H.%	F.H.
30	65%	34%	0	0
31	45%	54%	19%	7%
32	35%	64%	26%	8%
33	17%	78%	44%	17%
34	49%	47%	16%	34%
35	0	95%	72%	27%
36	13%	86%	?	?
37	31%	66%	3%	0
38	0	92%	?	?
39	32%	6%	4%	13%
40	0	90%	?	?
41	96%	68%	42%	15%
42	8%	56%	35%	10%
43	8%	63%	55%	14%
44	6%	83%	18%	10%
45	0	84%	51%	15%
46	30%	69%	34%	16%
47	0	92%	?	?
48	0	86%	?	?
49	3%	53%	32%	14%
50	0	92%	?	?
51	3%	81%	60%	20%
52	10%	51%	34%	12%
53	5%	83%	?	?
54	5%	84%	52%	20%
55	0	92%	?	?
56	22%	66%	6%	11%
57	50%	33%	0	15%
58	42%	57%	8%	7%
59	0	100%	62%	31%
60	45%	50%	68%	36%
61	0	67%	16%	16%
62	0	80%	84%	16%
63	93%	0	0	6%
64	0	80%	50%	40%
65	0	73%	96%	23%
66	6%	13%	60%	26%

NO.	NORMAL%	I.C.H.%	S.M.H.%	F.H.%
67	0	60%	100%	0
68	16%	40%	60%	16%
69	22%	55%	22%	11%
70	0	52%	76%	29%
71	33%	66%	0	22%
72	0	15%	85%	15%
73	0	0	0	0
74	32%	67%	11%	20%
75	0	66%	53%	13%
76	0	60%	40%	40%
77	15%	85%	?	?
78	0	0	66%	33%
79	0	84%	78%	0
80	0	76%	100%	0
81	0	88%	77%	33%
82	9%	90%	?	?
83	9%	76%	66%	23%
84	9%	90%	?	?
85	30%	70%	0	6%

TABLE-4

PERCENTAGE OF NORMAL AND ABNORMAL AIR CELLS OF **L.** TEMPORAL BONE OF 55 CASES OF TYPICAL DROWNING.

CASE No.	TOTAL AIR CELLS	NORMAL %	I.C.H. %	S.M.H. %	F.H. %
2	100	11%	62%	12%	23%
4	250	0	91%	85%	13%
5	100	5%	50%	67%	43%
6	45	8%	77%	0	13%
7	70	35%	35%	21%	7%
8	50	40%	36%	20%	36%
9	52	67%	19%	19%	23%
10	122	1%	54%	55%	42%
11	154	0%	97%	?	?
12	25	32%	48%	20%	24%
13	52	13%	76%	13%	19%
14	40	100%	0	0	0
15	43	46%	53%	18%	11%
16	48	41%	41%	?	?
17	55	18%	80%	?	?
18	40	10%	30%	30%	60%
19	66	34%	65%	15%	39%
20	82	4%	48%	?	?
21	101	0	71%	38%	61%
22	39	30%	69%	20%	43%
23	54	7%	92%	18%	18%
24	77	0	90%	?	?
25	80	0	90%	?	?
26	60	6%	90%	?	?
27	80	25%	55%	46%	15%
28	137	0	100%	69%	30%
29	40	87%	12%	0	0
30	100	20%	35%	58%	23%
31	82	48%	51%	0	6%
32	65	36%	49%	18%	12%
33	33	0	93%	69%	30%
35	89	17%	82%	43%	35%

CASE NO.	TOTAL AIR CELLS	NORMAL %	I.C.H. %	S.M.H. %	F.H. %
36	60	8%	91%	58%	16%
37	75	4%	86%	96%	0
38	?	?	?	?	?
39	60	13%	86%	?	?
40	43	37%	62%	27%	0
41	83	6%	93%	?	?
42	73	30%	47%	19%	10%
43	143	5%	94%	?	?
44	79	92%	2%	2%	6%
45	88	9%	76%	47%	22%
46	78	8%	85%	21%	15%
47	107	0	91%	56%	14%
48	80	22%	65%	35%	12%
49	112	0	89%	?	?
50	99	0	89%	?	?
51	131	3%	59%	43%	15%
52	87	0	91%	?	?
53	73	6%	78%	58%	16%
54	84	9%	64%	44%	14%
55	72	9%	69%	?	?
56	85	4%	84%	63%	21%
57	96	0	100%	?	?

TABLE-5

PERCENTAGE OF MAIN PATHOLOGICAL ABNORMALITIES OF THE AIR CELLS OF TEMPORAL BONE IN BATHTUB DROWNING CASES.

CASE	NORMAL%	I.C.H.%	S.M.H.%	F.H.%
9	53.3%	5%	?	?
	67.3%	19.2%	?	?
13	-	-	-	-
	13.4%	76.9%	13.4%	19.2%
14	64.2%	17.8%	7.1%	17.8%
	100%	0	0	0
16	52.6%	47.3%	?	?
	41.6%	41.6	?	?
29	80%	0	10%	20%
	87.5%	12.5%	0	0
43	96.1%	3.8%	3.8%	0
	92.4%	2.5%	2.5%	6.3%
77	50.9%	33.9%	0	15%

TABLE-6

TOTAL NUMBER OF AIR CELLS AND PERCENTAGE OF AIR CELLS AFFECTED BY I.C.H., S.M.H., AND F.H., IN CASES OF DEATH BY IMMERSION .

CASE	TOTAL	NORMAL%	I.C.H.%	S.M.H.%	F.H.%
1A	59	89%	0	3%	6%
	60	83%	0	3%	13%
2A	50	80%	0	10%	14%
	44	87%	0	0	0
3A	66	89%	0	0	10%
	74	91%	0	0	8%
4A	32	93%	0	0	6%

OTHER CASES RECOVERED FROM WATER, DIED FROM CAUSES OTHER THAN DROWNING OR IMMERSION.

5A	80	100%	0	0	0
	70	100%	0	0	0
6A	113	76%	11%	5%	11%
	121	72%	8%	5%	13%
7A	105	17%	61%	11%	20%
	68	36%	51%	4%	26%
8A	116	8%	56%	35%	10%
	112	6%	65%	45%	13%

TABLE-7

DESCRIPTION OF CASES RECOVERED FROM WATER.

DATA	NUMBER	I.C.H.	
		+ve.	-ve.
IMMERSION SYNDROME	4	0	4
OTHER CAUSES OF DEATH	4	3	1
TYPICAL DROWNING	85	81	3 Unilat

TABLE-8

DESCRIPTION OF NUMBER OF TEMPORAL BONES EXAMINED IN CASES OF TYPICAL DROWNING.

DATA	No.	I.C.H.	
		+ve.	-ve.
R.TEMPORAL BONE	85	79	2
L.TEMPORAL BONE	55	53	1
BILATERAL	55	51	3 Bilat.
Unilat.			

* 4 R.side temporal bones were badly pnuematised.
* 1 L.side temporal bone was badly pnuematised.
* In one case,the bad pnuematization was on both sides of the bones.

TABLE-9

DESCRIPTION OF GRADESOF INTRACAVITARY HAEMORRHAGE IN CASES OF TYPICAL DROWNING.

DATA	No.	GRADE		
		I	II	III
R.TEMPORAL BONE				
	2	-	-	-
	6	+	-	-
	12	-	+	-
	61	-	-	+
L.TEMPORAL BONE				
	1	-	-	-
	3	+	-	-
	10	-	+	-
	38	-	-	+

TABLE-10

DESCRIPTION OF GRADESOF SUBMUCOSAL HAEMORRHAGE IN CASES OF DROWNING.

DATA	No.	GRADE		
		I	II	III
R.TEMPORAL BONE				
	5	-	-	-
	14	+	-	
	17	-	+	-
	27	-	-	+
L.TEMPORAL BONES				
	4	-	-	-
	12	+	-	-
	12	-	+	-
	10	-	-	+

TABLE-11

DESCRIPTION OF FOCAL HAEMORRHAGE IN CASES OF TYPICAL DROWNING

DATA	No.	GRADE		
R.TEMPORAL BONE		I	II	III
	7	-	-	-
	36	+	-	-
	20	-	+	-
	0	-	-	+
L.TEMPORAL BONE				
	4	-	-	-
	19	+	-	-
	13	-	+	-
	2	-	-	+

TABLE-12

DESCRIPTION OF DEGREE OF SEVERITY OF INTRACAVITARY HAEMORRHAGE, SUBMUCOSAL HAEMORRHAGE AND FOCAL HAEMORRHAGE OF RIGHT TEMPORAL BONES IN CASES OF TYPICAL DROWNING.

DATA	No.	DESCRIPTION SEVERITY
I.C.H.	25	SEVERE
	24	MODERATE
	30	MILD
S.M.H.	15	SEVERE
	14	MODERATE
	29	MILD
F.H.	4	SEVERE
	14	MODERATE
	40	MILD

TABLE 13

NUMBER AND PERCENTAGE OF NORMAL AND ABNORMAL AIR CELLS OF 17 CASES OF HANGING.

CASE	TOTAL	NORMAL%	I.C.H.%	S.M.H.%	F.H.%
1	93	91%	0	0	8%
	88	92%	5%	4%	3%
2	106	23%	64%	38%	24%
	59	32%	56%	27%	71%
3	42	100%	0	0	
	0				
	44	100%	0	0	0
4	50	84%	4%	0	0
	43	93%	2%	0	4%
5	59	22%	35%	16%	28%
	33	51%	27%	6%	9%
6	51	43%	21%	33%	13%
	-	-	-	-	-
7	97	62%	18%	12%	24%
	102	39%	21%	17%	30%
8	59	37%	62%	25%	8%
	48	31%	62%	20%	50%
9	37	86%	5%	5%	8%
	88	56%	25%	1%	26%
10	96	12%	62%	14%	22%
	65	35%	26%	6%	35%
11	62	69%	19%	20%	8%
	36	91%	5%	2%	5%

CASE	TOTAL	NORMAL%	I.C.H.%	S.M.H.%	F.H.%
12	90	50%	11%	13%	13%
	70	57%	0	0	30%
13	85	29%	23%	23%	30%
	91	83%	9%	6%	9%
14	96	91%	0	2%	6%
	75	93%	0	1%	4%
15	84	57%	11%	14%	28%
	65	50%	35%	9%	30%
16	99	66%	12%	10%	28%
	-	-	-	-	-
17	84	53%	11%	9%	23%
	80	56%	16%	13%	30%

TABLE 14

NUMBER OF TOTAL AIR CELLS WITH THE PERCENTAGE OF ABNORMAL AIR CELLS OF TEMPORAL BONE OF ASPHYXIAL CASES.

CASE	TOTAL	NORMAL%	I.C.H.%	S.M.H.%	F.H.%
33	64	68%	12%	0	18%
34	24	100%	0	0	0
35	35	91%	0	0	8%
36	19	63%	10%	10%	26%
37	19	42%	42%	0	15%
38	72	0	11%	58%	41%
39	20	85%	0	0	15%
40	110	54%	9%	16%	38%
42	63	68%	4%	4%	9%
43	40	60%	22%	7%	32%

TABLE-15

NUMBER OF TOTAL AIR CELLS WITH THE PERCENTAGE OF ABNORMAL AIR CELLS OF TEMPORAL BONE IN 15 ISCHAEMIC HEART DISEASE CASES.

CASE	TOTAL	NORMAL%	I.C.H.%	S.M.H.%	F.H.%
18	91	73%	5%	10%	15%
19	113	97%	0	0	3%
20	23	86%	0	0	3%
21	72	97%	0	0	2%
22	76	92%	0	2%	4%
23	81	96%	0	1%	2%
24	18	100%	0	0	0
25	60	90%	3%	1%	5%
26	74	91%	0	2%	4%
27	28	96%	0	0	3%
28	45	100%	0	0	0
29	37	94%	0	2%	0
30	33	84%	0	3%	12%
31	53	94%	0	0	5%
32	77	90%	0	2%	6%

TABLE-16

PERCENTAGE OF NORMAL AND ABNORMAL AIR CELLS OF TEMPORAL BONE IN CASES OF INTRACRANIAL HAEMORRHAGE

CASE	TOTAL	NORMAL%	I.C.H.%	S.M.H.%	F.H.%
44	78	38%	34%	32%	16%
45	99	34%	45%	13%	13%
46	75	0	69%	66%	2%
47	50	30%	70%	0	6%
48	78	48%	32%	25%	15%
CASES OF CARBON MONOXIDE POISONING					
49	132	4%	87%	52%	24%
50	80	5%	70%	67%	27%
51	25	92%	0	0	12%
52	83	85%	3%	16%	15%
53	66	65%	12%	4%	18%

TABLE 18

DESCRIPTION OF GRADES OF SEVERITY OF EACH TYPE OF PATHOLOGICAL ABNORMALITY SEEN IN AIR CELLS OF R.TEMPORAL BONES OF 85 CASES OF TYPICAL DROWNING.

PERCENTAGE %	NORMAL	I.C.H.	S.M.H.	F.H.
0-10%	48	4	14	18
11-20%	8	4	5	25
21-30%	5	1	6	9
31-40%	7	8	7	11
41-50%	7	3	4	0
51-60%	2	11	10	0
61-70%	2	10	6	0
71-80%	1	12	5	0
81-90%	1	17	2	0
91-100%	0	11	4	0
TOTAL *	81	81	63	63

* EXCLUDING CASES WITH BAD PNUEMATIZATION = 4
EXCLUDING CASES WITH ADVANCE DEGREE OF AUTOLYSIS (FOR S.M.H.& F.H.) = 18

TABLE 19

DESCRIPTION OF SEVERITY OF EACH TYPE OF PATHOLOGICAL ABNORMALITY SEEN IN AIR CELLS OF LEFT TEMPORAL BONES IN CASES OF TYPICAL DROWNING.

PERCENTAGE %	NORMAL	I.C.H.	S.M.H.	F.H.
0-10%	43	2	5	14
11-20%	4	2	11	9
21-30%	4	1	4	7
31-40%	5	4	2	3
41-50%	2	5	6	3
51-60%	0	5	5	1
61-70%	1	7	4	1
71-80%	0	6	0	0
81-90%	1	10	0	0
91-100%	2	10	1	0
TOTAL	52	52	38	38

* EXCLUDING CASES WITH ADVANCED =14
DEGREE OF AUTOLYSIS (FOR S.M.H.& F.H.)

air cells, whilst others showed only mild or moderate degrees of haemorrhage. For this reason, it was taken into consideration to assess the severity of haemorrhage quantitatively as well as qualitatively, in order to reach a definite conclusion about the severity of the haemorrhage. To be more precise, the air cells were recorded in their actual number seen microscopically to make the comparison easy between the normal and affected air cells (Tab.4). These figures are presented as percentages of the total number of air cells of both right and left temporal bones. Tables 18 and 19 showing the number of cases categorized as a particular type of haemorrhage with the percentage of air cell involvement of each type, irrespective of the severity of the haemorrhage. The percentages are arranged from 10% to 100% (in 10 categories).

3.1.2. MUCOSAL LINING OF TYMPANOMASTOID SPACES :

The lining epithelium of the middle ear and mastoid air cells appeared normal. Oedema of the lamina propria was a striking features in 66 cases. It was not clearly prominent in 5 cases, and in 6 other cases it was absent.

The mucosa which showed oedematous change was clear from any cellular infiltrate. The lamina propria which failed to show oedema, adhered closely to the periosteum and capillaries were seen as small dots. The vessels within the lamina propria were engorged and even the mucosa investing the ossicles, muscle tendons and nerve sheath showed this phenomenon. These congested vessels

within the lamina propria were sometimes surrounded by petechial haemorrhage.

The most striking microscopical feature within the lining mucosa, was a more severe type of haemorrhage involving all or most of the mucosa of the middle ear (Fig.5) and air cells (Fig.4). It is called submucosal haemorrhage to differentiate it from the focal type which consists of minute extravastion of red blood cells.

It is not uncommon to find both I.C.H. and S.M.H. in the same degree and grade of severity in many cases of drowning (Tabs.4, 9-12). It is considered a separate clinical entity from I.C.H.

Submucosal haemorrhage, also, was classified into degrees of mild, moderate and severe according to the area of involved mucosa with S.M.H. in each air cell. The appearance of S.M.H. was an easily recognizable sign in fresh bodies. Cases with advanced autolysis presented some difficulty in distinction between autolytic mucosa of the air cells and the haemolysed red blood cells within the mucosa. To obviate this we omitted the 18 of the right and 15 of the left temporal bones, which showed some autolysis, from our results (Tabs.4, 18 and 19).

The degree of S.M.H. in the right temporal bones showed the following findings, 5 cases (7%) were negative, 29 cases (46%) mild, 14 cases (22%) moderate and 15 cases (23%) severe. The 54 left temporal bones showed, 17 cases (44%) mild, 12 cases (31%) moderate, 5 cases (13%) severe,and only one negative. The above figures showed

that around 45% of right side and 44% of the left one have been affected by a moderate to severe degree of S.M.H. If we look at Tables (10) the Grade III lesion has been shown in around 42% of the right side temporal bones, and 26% of the left ones. In Table (18) the number of cases involved with any particular percentage of the air cells was more or less equally distributed, except two sets of percentage showed high number of cases, and these are 14 cases with 10%, 5 of them negative, and a second set with 10 cases of 51-60% involvement.

Nearly in all the series, the degree of S.M.H. was similar on both temporal bones, even to the grade of involvement of air cells being nearly identical on both sides, except in a very few cases which showed no haemorrhage in one side while the other side was positive. These anomalies are explained on basis of poor pneumatization and this appears a satisfactory reason, but in other examples no cause could be attributed to explain the difference between right and left bones.

As far as pathological changes within the lining mucosa are concerned. We detected other minute haemorrhages, perivascular extravastion of red blood cells around engorged blood vessels. It is called focal haemorrhage to differentiate it from S.M.H., which could mask the focal one if they occurr together in the same mucosa of the air cell lining. For that reason and in order to grade the severity of focal haemorrhage, we have taken in consideration those air cells with only this type of

haemorrhage. After omission of cases with autolytic changes, 28 specimens of right air cells showed a mild degree of F.H. while 14 and 4 specimens showed moderate and severe degrees respectively (Tabs.4 and 11). There were 5 specimens without such haemorrhage in their mucosa of either mastoid area or middle ear; while 4 other cases were blamed by poor pneumatization as a cause of absence of F.H.

On the left side, the following results were observed, 17 specimens showed mild degree, 8 moderate and 2 severe degree (Tab.4). There were 4 negative cases and one case with poor pneumatization (Tab.8). The interesting feature, regarding the grade of F.H. was the failure to find any specimen in the right temporal bones with involvement of more than 50% of its air cells with F.H. i.e. Grade III. In the left temporal bones there were only 2 specimens (5%) with Grade III changes (Tab.11). These figures of F.H., which represent a mild degree of severity do not permit the explanation of congestion as a cause of haemorrhage in drowning.

Concerning the similarity in degree or grade of F.H. in both sides, there were 7 cases showing no F.H. in one side of the bone, while positive results were detected in the other side. The difference of F.H. in both sides was explained according to the criterion of poor pneumatization in one case only, while in the other 6 no reason could be adduced. However,the rest of the cases shared the same criteria of similarity in degree and grade of this type of haemorrhage.

3.1.3. INNER EAR EXAMINATION :

Generally speaking there were no important morbid changes seen in the inner ear. The neuroepithelium of the vestibular labyrinth and cochlea were in an excellent state of preservation, in nearly all the fresh cases, and mild to moderate autolytic changes were seen in the putrefied bodies. The organ of Corti was well persevered. The marginal cells of the stria vascularis were unremarkable in all cases. The semicircular canals, crista and macula were all normal. However these structures showed some degree of engorgement of their blood vessels, but without petechial haemorrhage.

Petechiae were detected in the internal acoustic meatus in 9 cases of typical drowning usually the haemorrhage being presented as tiny spots of extravasated blood within the vestibulo-cochlear nerve and its sheath. One case showed haemorrhage within the vestibulo-cochlear ganglion while another, both the modiolus and vestibulo-cochlear nerve showed the haemorrhage. In these cases, no pathological cause or relevant factor could explain the occurrence of haemorrhage in the internal acoustic meatus and its structures, except Case No. 20 which showed a fracture of the left side of the skull with underlying damage of the brain.

In the inner ear one could say quite confidently that the absence of pathological abnormalities, apart from congestion of blood vessels, is the usual finding in cases of drowning.

3.2. IMMERSION SYNDROME :

As already mentioned, 4 bodies recovered from water which initially thought to have drowned, a diagnosis subsequently excluded on the basis of the gross post-mortem findings. Furthermore, this presumption of the diagnosis of immersion syndrome, has been substantiated by the failure to detect I.C.H. in any of air cell of these cases.

The normal air cells were over 80% of the total number in any one of the 4 cases. F.H. was shown also to be very mild and in very few air cells in nearly all the cases (Tab.6). No other pathology was detected in the tympanomastoid area or inner ear structures.

3.3. OTHER CASES RECOVERED FROM WATER :

The lack of the classical signs of drowning, and the presence of other natural or unnatural causes of death in 4 bodies recovered from water. The diagnosis was reached as follows;

Case No 5A; inhalation of blood and multiple injuries due to fall from height.

Case No 6A, lorazepam intoxication, accidental hypothermia and ischaemic heart disease.

Case No 7A, accidental hypothermia, acute alcohol intoxication and hypertensive heart disease.

Case No 8A, pulmonary haemorrhage and oedema, inhalation of stomach content, due to an accident in swimming pool.

Case No 5A was a 40 year old male. His body was recovered from a quarry and initially he was thought to have died from drowning. At necropsy, there was bilateral plural adhesions with a fair amount of blood in the bronchi, but no sign of water inhalation. Examination of the head revealed comminuted fracture of the skull with a linear fracture of the right middle cranial fossa accompanied by laceration of the cerebrum and cerebellum and mild subarachnoid haemorrhage. Such findings were enough to diagnose the case as death due to inhalation of blood and multiple injuries due to a fall from a height. However, this diagnosis was challenged on the grounds of positive findings of diatoms in the lungs and bone marrow of the sternum. However, temporal bone examination substantiated the first diagnosis i.e. inhalation of blood with multiple injuries. The tympanomastoid area was normal, and haemorrhage could not be found either in middle ear or in the well penumatized air cells. These findings applied to all types of intracavitary, submucosal and focal haemorrhage.

The Case No. 6A was a 74 years old male. He was found lying semi-conscious on a grass bank, only partly dressed with the remainder of his clothing on the ground nearby. He was taken to hospital but was dead on arrival. Post-mortem examination showed that he had substantial ischaemic heart disease with evidence of an old myocardial infarction and occlusion of one of the coronary arteries. By itself, this degree of heart disease was adequate to cause death. He also showed

signs of hypothermia. The most likely explanation for his death was initially thought to be an overdose of lorazepam tablets, and that while unconscious he developed hypothermia. There were no signs of inhalation of water, as the plural cavities were clear and dry. The air passages were clear and both lungs were essentially normal. However histopathological examination of the temporal bones revealed a mild degree of I.C.H., S.M.H. and F.H. but not sufficient for a diagnosis of drowning. Failure to show inhalation of water might be due to chronic bronchitis and emphysematous changes that founded in the victim who already suffered from kyphoscolosis of thoracic vertebra with pigeon chest deformity.

The Case No 7A was a 60 years old male. He was found dead, lying face down in a water filled ditch near to where his possessions were found. Post-mortem examination showed some changes on the surface of the body suggestive of hypothermia e.g. pink blotchy areas of the knees and elbows. Internally, some, though, not classical evidence of drowning were found. He also had an enlarged heart and at the time of his death, was considerably intoxicated by alcohol. Microscopical examination of the tympano-mastoid area showed 61.9% and 51.4% of right and left temporal bone respectively had been affected by I.C.H.. Submucosal haemorrhage and F.H. were also detected but in lesser degree and grade of severity. Congestion of blood vessels and obvious oedematous changes of the lamina propria were also observed. No

other abnormality was found outside the tympanomastoid area.

Case No 8A, was a 28 years old male. He was seen lying at the bottom of swimming pool. He was taken out of the water but despite vigorous attempts at resuscitation he died soon afterwards. Post-mortem examination showed that he had died as the result of inhaling vomit into the lung, this having produced massive pulmonary haemorrhage. He did not die primarily of drowning although it remains a possibility that for some reason he did in fact aspirate water and partially drown and that this and/or the attempts to resuscitate him caused him to vomit and subsequently inhale this into his lungs. The microscopical appearance of the temporal bone however would suggest death due to drowning. Moderate I.C.H. has been found in 56% and 65.1% of right and left air cells of the temporal bones respectively with a moderate grade of S.M.H. on both sides. There was considerable engorgement of the blood vessels with focal haemorrhage. The lamina propria showed oedematous change.

3.4. BATHTUB DROWNING :

In cases of bathtub drowning we have tried to assess the severity of the pathological changes in the tympanomastoid area to reach a better understanding of the pathogenesis of intrapyramidal haemorrhage in cases of drowning in shallow water.

Six bodies were found dead in bath-tubs which

contained water at the time of their discovery, whilst in Case No. 16 discovered in a advanced stage of decomposition, lying above his mother who had been strangled before she was placed in the bath, the bath was empty. The diagnosis in this case, drowning, was based on the exclusion of other causes of death as well as on the basis of circumstantial evidence. In other cases, the diagnosis was made according to post-mortem findings.

Most of them were under the influence of alcohol or other drugs at the time of being immersed in the water, but in Cases No 43 and 76 no detectable drug could be found.

Histopathological examination showed grade I intracavitary haemorrhage in 4 of the cases which was mild in 3 , and severe in the other one (Tab.5). Also, we found the left bone of Case No 14 and the right bone of cases No 16 and No 76 revealed Grade II haemorrhage with moderate and mild degree haemorrhage respectively.

In the last Case, No 18, the right temporal bone was penumatized and has not been examined fully, the left bone showed Grade III (76.9%) haemorrhage with a moderate degree of severity.

Submucosal haemorrhage could not be appreciated as in the other cases of drowning The findings of severe natural disease or high levels of alcohol or other drugs were not much help in explaining the scarce in grade and degree of haemorrhage.

3.5. HANGING :

For better understanding of the pathogenesis of intrapyramidal haemorrhage, conditions that cause severe congestion with hypoxia have been investigated on the same lines as in drowning. This group comprised 17 cases of hanging victims, with age range of age was 18-70 years (Tab.2).

The usual mode of hanging, in most of cases was achieved by a rope tied around the neck and in turn attached to the top of a door, kitchen pulley, or to the handle of a door after being hooked over the top of the door. Therefore the bodies in these cases were in a position of partial suspension. They included cases No. 1, 3, 5, 8, 9, 10, 11, 13, 14, 15 and 16. In other 3 cases the bodies were completely suspended above the floor. Though the cause of death in all 17 cases was certified as hanging, in 2 cases No 8, and 13, a combination of strangulation and hanging had been anticipated, since the rope had been looped around the neck more than once in one case and the finding of a polythene bag positioned over the head of the deceased in the other.

However, necropsy findings differed from one case to another. Petechial haemorrhages were found in the conjunctivae or sclera in 3 victims, while internally no petechia could be seen in one single case apart from dubious sub-endocardial haemorrhage in one case. The diagnosis was straight forward from specific anatomical

findings in the neck and from circumstantial evidence. Alcohol analysis was positive in 6 cases in which the level was more than 100mg/100 ml blood in 5 of them. In all the 17 cases, the right temporal bones were examined and were fully penumatized. while only 15 left bones were considered in the analysis, because of poor pneumatization in one bone and technical difficulty in the other.

3.5.1. INTRACAVITARY HAEMORRHAGE :

Variable degrees of I.C.H. were obtained in most cases of hanging, only the right bone in one case and the left in another were negative. Two further cases failed to show any haemorrhage on both sides. In the positive cases, the haemorrhage into the air cells was mild in degree and grade in most cases.

As shown in Tables.13 and 20 the severe grade of haemorrhage (i.e. Grade III) was found in 3 specimens of the right bone and 2 of the left bone; while Grade II was detected in 3 right bones and 5 left ones. The rest of both sides were either of mild grade or failed to show any I.C.H. From those figures, it is concluded that in the majority of the hanging cases, the occurrence of I.C.H. would be less than 50% in the total number of air cells. The number of normal air cells would be expected to be high, and the degree of haemorrhage found to be less severe than in drowning. Table 20 shows 10 cases with mild severity of both of right and left temporal

bones among 14 and 12 positive specimens of these bones respectively. The most outstanding feature in these conditions was the engorgement of blood vessels throughout the external, middle and inner ears. No petechial haemorrhage was found outside the tympanomastoid region.

Oedema of the lamina propria was detected in some cases but in a mild degree.

Inner ear structures were intact and unremarkable except for the engorgement of the blood vessels which could be demonstrated in most areas of the inner ear, and was without accompanying petechia.

Generally, the lack of type and degree of haemorrhage seen in cases of drowning would throw doubts about the congestion of blood vessels and increased venous blood pressure as being causes of intrapyramidal haemorrhage.

3.6. ASPHYXIA :

Asphyxial cases other than hanging have been examined to see the effect of the mechanical obstruction of the respiratory passages and of anoxia of the middle ear and air cells as possible causes of haemorrhage. Ten cases of asphyxia were examined. The right temporal bone on the lines already outlined for investigation of haemorrhage in the tympanomastoid area.

The specimens were derived from 5 male and 5 female patients with ages from 7 weeks to 71 years. The causes of asphyxia were due to choking on food, inhalation of gastric content, suffocation, partial asphyxia due to

mechanical constriction of the neck, and epilepsy. (Table 2).

Intracavitary haemorrhage was found in 7 cases and negative in 3 (Table 14). Five cases out of the positive 7 cases showed only grade I haemorrhage, while the other two showed I.C.H. of grade II (Table 22). However, the degree of haemorrhage in all positive cases was mild.

The ages of two of the negative cases were 7 weeks and 10 months, and they showed some degree of pneumatization of the mastoid area, though these air cells failed to show any I.C.H. Both infants died from asphyxia due to inhalation of gastric content.

Other haemorrhages (S.M.H.) were negative in 5 cases, while all the mild degree positive cases showed grade I involvement of the air cells, (except one case with Grade III) who was a 3 years old female child died from cerebral oedema and partial asphyxia due to mechanical constriction of the neck.

Concerning F.H. there was only one negative case the rest showed mild F.H. either grade I or II involvement. (Table 22).

The lamina propria showed some oedema especially in cases No 1, 3 and 5. Congestion of blood vessels was a feature in most of the cases.

3.7. INTRACRANIAL HAEMORRHAGE:

Five cases represented three main different causes of intracranial haemorrhages including skull fracture,

TABLE 20

DESCRIPTION OF GRADES OF SEVERITY OF EACH TYPE OF PATHOLOGICAL ABNORMALITY IN THE AIR CELLS OF TEMPORAL BONES IN 17 CASES OF HANGING.

PERCENTAGE %	NORMAL		I.C.H.		S.M.H.		F.H.	
	R.	L.	R.	L.	R.	L.	R.	L.
0-10%	0	0	5	7	7	11	7	7
11-20%	1	0	6	1	6	3	2	0
21-30%	3	0	2	4	2	1	8	4
31-40%	1	4	1	1	2	0	0	1
41-50%	2	1	0	0	0	0	0	2
51-60%	2	4	0	1	0	0	0	0
61-70%	3	0	3	1	0	0	0	0
71-80%	0	0	0	0	0	0	0	1
81-90%	2	1	0	0	0	0	0	0
91-100%	3	5	0	0	0	0	0	0
TOTAL	17	15	17	15	17	15	17	15

TABLE 21

DESCRIPTION OF GRADES OF SEVERITY OF MAIN PATHOLOGICAL CHANGES IN AIR CELLS OF TEMPORAL BONE IN CASES OF ISCHAEMIC HEART DISEASES.

PERCENTAGE%	NORMAL	I.C.H.	S.M.H.	F.H.
0-10%	0	15	15	13
11-20%	0	0	0	2
21-30%	0	0	0	0
31-40%	0	0	0	0
41-50%	0	0	0	0
51-60%	0	0	0	0
61-70%	0	0	0	0
71-80%	0	0	0	0
81-90%	4	0	0	0
91-100%	11	0	0	0
TOTAL	15	15	15	15

TABLE 22

THE DESCRIPTION IN CASES OF ASPHYXIA.

PERCENTAGE%	NORMAL	I.C.H.	S.M.H.	F.H.
0-10%	1	6	8	3
11-20%	0	2	1	3
21-30%	0	1	0	1
31-40%	0	0	0	2
41-50%	1	1	0	1
51-60%	2	0	1	0
61-70%	3	0	0	0
71-80%	0	0	0	0
81-90%	1	0	0	0
91-100%	2	0	0	0
TOTAL	10	10	10	10

subarachnoid haemorrhage and cerebral haemorrhage. These were selected for temporal bone examination (Table 2). The diagnosis of these cases was straightforward from post-mortem findings which gave no doubt about the cause of death. These cases were selected to compare the histopathological appearance of the tympanomastoid area.

However, microscopy revealed all types of haemorrhage I.C.H., S.M.H. and F.H. in all the cases except Case No 4 which failed to show S.M.H. Intracavitary haemorrhage, could not be distinguished from that of the drowning cases, so far as the degree and grade of haemorrhage were concerned (Table 16).

Variable degrees of haemorrhage (I.C.H.) were detected in more that 30% of the air cells of every case which included 3 cases with Grade II and 2 cases with Grade III.

S.M.H. affected 4 cases with different grades of air cells involvement as well as different degree of severity. This differs from what was seen with F.H. where only a few air cells were involved with usually a mild degree of haemorrhage (Tab.16).

3.8. CARBON MONOXIDE POISONING :

Naked-eye examination of the temporal bones often discloses abnormal dark brownish-red discoloration of the middle ear in cases of carbon monoxide poisoning.

Hence, the opportunity existed for the examination of the effects of anoxia in poorly supported blood vessels

like those of the mucosa of the middle ear and mastoid air cells and also to investigate the nature of this abnormal discoloration of petrous bone.

I.C.H. was negative in one case while in other cases mild haemorrhage was seen in very few air cells. Surprisingly, 2 cases with macroscopical discoloration of the bone showed severe degrees of haemorrhage in 87.8% of the air cells in one case and 70% of the air cells in the other. Such severity of I.C.H. was also found with S.M.H. in 2 cases. The other 2 cases revealed mild S.M.H. in few air cells (Tab. 16).

F.H. involved less than 20% of the air cells in 3 cases,and was mild in degree. In 2 other cases, F.H. was detected in 24.2% of the air cells in one case and 27.5% of the air cells in the other, both were in moderate degree. No other abnormality was detected, apart from the obvious engorgement of blood vessels distributed throughout the temporal bones, but petechia were absent in the area outside tympanomastoid spaces.

3.9. ISCHAEMIC HEART DISEASE :

This group of 15 patients represented death due to acute circulatory failure and sudden anoxia due to severe ischaemic heart disease. Severe coronary artery atheroma with or without recent thrombosis was the usual finding in most of the cases which belonged to the age group between 42-70 years, (except in one case aged 7 days who died from complex congenital heart disease) (Tab.2).

Histopathological examinations of the temporal bones

revealed negative findings of the 3 types of haemorrhage i.e. I.C.H., S.M.H. and F.H. in 13, 7 and 3 cases respectively (Tab.15 and 21). Most of the cases with ischaemic heart disease were characterised by petechial haemorrhage in its focal form in the area of the mastoid air cells, and if any type of haemorrhage had been found it would have occupied only very few air cells (Tabs. 15 and 21).

Congestion of the capillary blood vessels in the lamina propria of the mastoid air cells, muscles, nerve sheath and even sometimes the inner ear was detected but without extravasation of blood. Oedema of the lining mucosa was usual in most cases, but variable from one case to another and even in the same case.

Inner ear structures were well preserved and showed no abnormality.

3.10. MISCELLANEOUS CASES :

In an effort to determine the overall incidence of tympano-mastoid haemorrhage in necropsy material, we examined 17 temporal bones obtained from bodies with different causes of death.

These included 3 cases of sudden infant death syndrome, 3 of liver diseases (either in form of hepatorenal failure or fatty liver), 2 cases of fire death, 3 cases of bronchopneumonia, 2 cases of drug poisoning (one with Chlormethiazole and other with Nitrazepam) and each case of the other 4 represented with one of the following

pathologies, large bowel obstruction, peritonitis, multiple injuries and rupture of a dissecting aneurysm of the aorta (Tab.2).

Only cases of hepatorenal failure and chlormethiazole poisoning showed some degree of dark brownish-red discoloration of the outer surface of the temporal bones. The same two cases showed mild to moderate I.C.H. in a moderate number of the air cells.

The figures of percentage of the air cells affected by all types of haemorrhages, in case No. 7, are showed in Table.17.

In the 3 cases of cot death, all histological sections showed normal features. There was well- formed bone marrow formation with vasodilation of all the blood vessels but no sign of haemorrhage.

The pneumatization was fairly well developed in the other cases but they showed unremarkable mucoperiostum with healthy intact blood vessels and were without evidence of haemorrhage except in very few air cells in few cases. No other abnormality was discovered.

4. DISCUSSION :

4.1. PNEUMATIZATION OF THE TEMPORAL BONE

Throughout the study of temporal bones of different ages and sexes, we have found a variable degree of pneumatization ranging from minimal air cell development to total involvement the temporal bone, which conforms with the results of other workers (72,108). The growth

pattern of pneumatization is thought to be controlled by heredity, environment, nutrition, bacterial infection and the adequacy of ventilation as determined by Eustachian tube function. Pneumatization is the result of a hollowing out process in which mesenchyme is resorbed to leave spaces. It begins in early fetal life and progresses during infancy and childhood to maturity.

Some of our cases especially in infants up to 10 months old, have shown poor pneumatization because of their air cells being still occupied by diploic bone marrow cells. Bones from infants below that age however sometimes showed a degree of pneumatization. While the size of the air cell system may be negligible during the first 6 months of life, it can still help in certain cases when the diagnosis depends on the pathological findings in the few air cells which are present.

Poor pneumatization has been also detected in the old age group which could be hereditary in nature, as the size of the air cell system is claimed to be genetically determined (179), or it might be the result of previous middle ear inflammatory diseases, as claimed by supporters of the environmental pneumatization theory (43). The size of the air system in most cases seemed to be asymmetrical, and such a result would favour the environmental theory of pneumatization which considers the phenomenon of asymmetry valid and logical Therefore we have chiefly considered the air cells of suitable size with easily recognizable mucous membrane in which any haemorrhage would be easily detected.

We discovered asymmetry not only between the sizes of air system in one side of the bone but also between the left and right side of the same patient. This is a predominant genetically determined phenomenon (43).

4.2. TEMPORAL BONE HAEMORRHAGE :

4.2.1. Macroscopical Examination :

The reliance on naked-eye examination of the temporal bones as an indication of intrapyramidal haemorrhage proved sometimes to be misleading sign. Severe types of haemorrhage usually presented as dark reddish-brown discoloration of the petrous pyramid just beneath the semi-translucent tegmen tympani. However in some cases, severe intrapyramidal haemorrhage could not be detected. This view was reached after finding 4 cases of immersion syndrome devoid of haemorrhage either naked-eye or microscopically. The dorsal approach might help in showing more clearly morbid changes in the middle ear and mastoid air system. It might even be diagnostic where coagulated blood and clear haemorrhage were seen in these areas leaving no doubt of intrapyramidal bleeding (Figs.1,2). But this is unusual, since macroscopical detection of I.C.H. and S.M.H. associated with different pictures e.g. petechiae, dark or faint brown discoloration, congested bone.

The difficulty was greater in the differentiation of the bone marrow air cells from haemorrhagic air cells, and this can be settled only by microscopical

examination. An interesting finding in cases other than drowning, was the failure to see any discoloration of the bone either on the outer surface or in the dorsal section, though variable degrees and grades of I.C.H. and S.M.H. might be detected microscopically. The explanation for this is obscure.

4.2.2. Intra-Cavitary Haemorrhage :

Examination of 81 right temporal bones from 85 cases of typical drowning, (excluding 4 specimens of badly penumatized bones) has showed free blood in the middle ear and mastoid air cells in percentage of 4.7%, 14.7% and 75.3% for grade I, II and III respectively. These results do not differ much from those of 54 left bones from the same group of cases with percentages of 5.5%, 19.2% and 73% for the same sequences of grades. That means more than 50% of air cells on either temporal bones in more than 75% of drowning cases did contain free blood in the tympano-mastoid area in the form of intracavitary haemorrhage (Fig.7). Free blood in these spaces was usually severe enough to involve about 60% of either temporal bone in moderate to severe degree.

These results generally confirm the idea of similarity in morbid changes of both bones so far as grade and degree of haemorrhage are concerned. Also, they indicated that examination of up to 5 histological sections from each temporal bone was adequate for studying the pathological changes in the different cases.

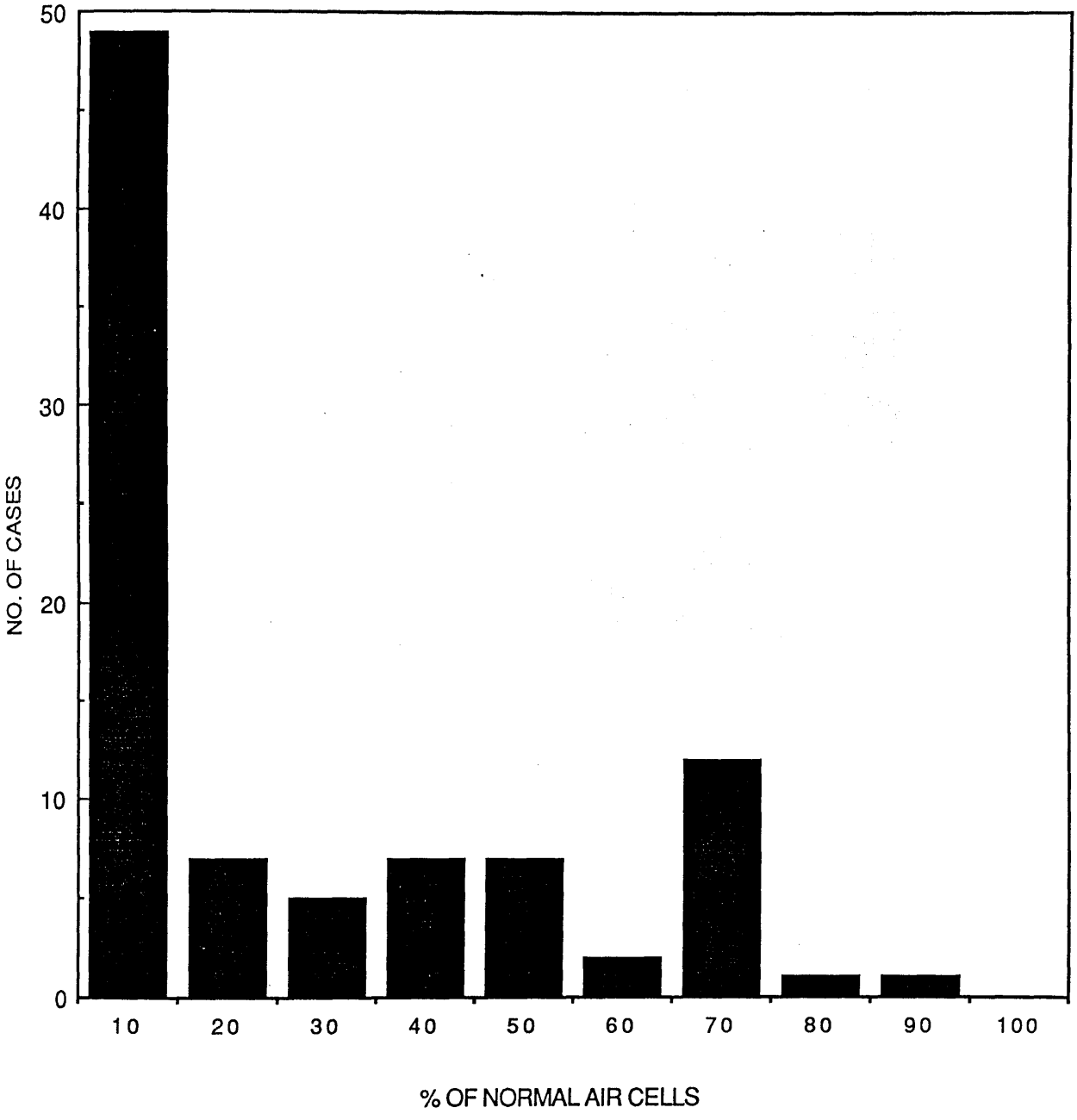

Fig. 6. Description of the percentage of normal air cells in drowning cases

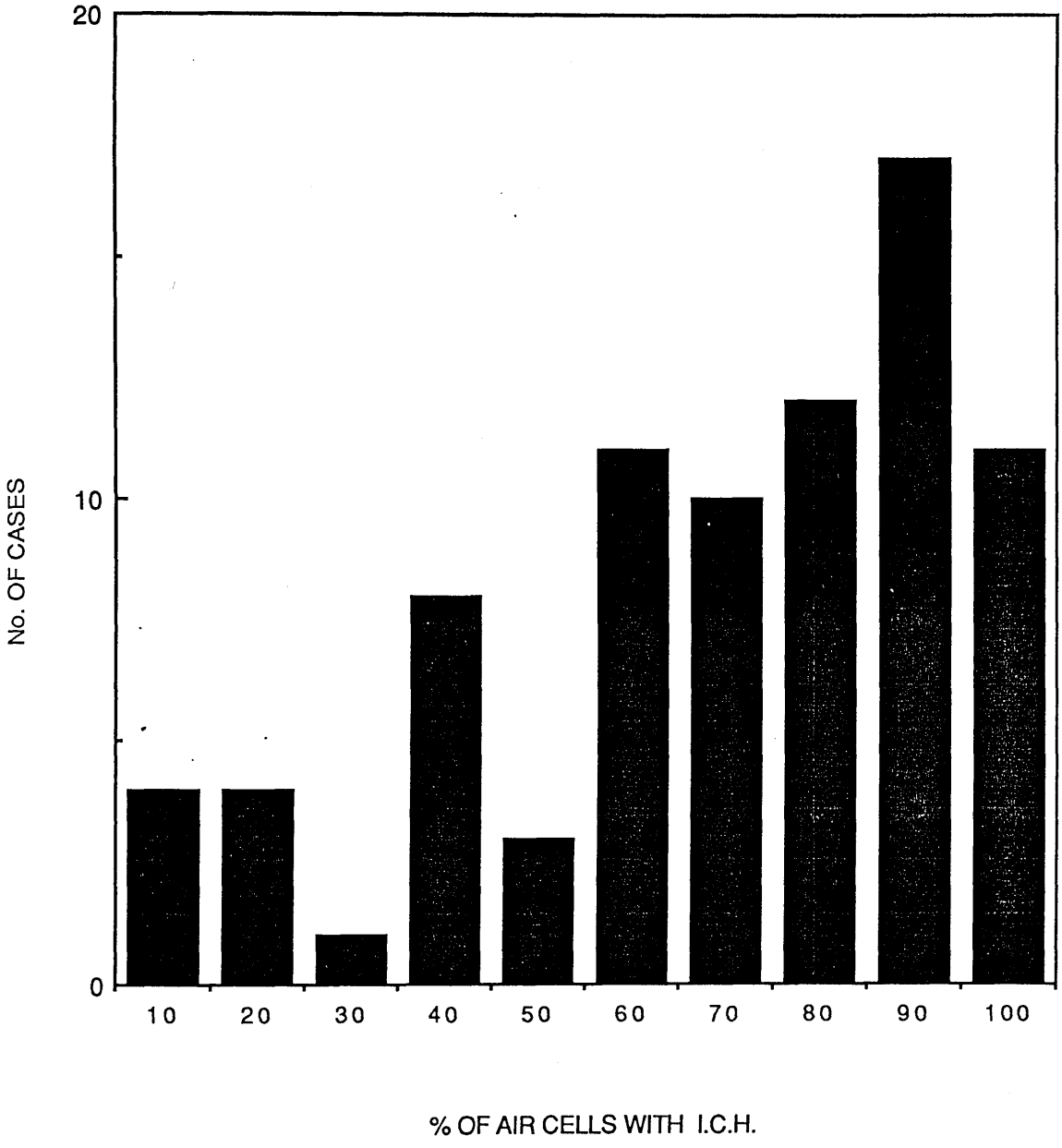

Fig. 7. Description of the percentage of the air cells affected by I.C.H. in drowning cases.

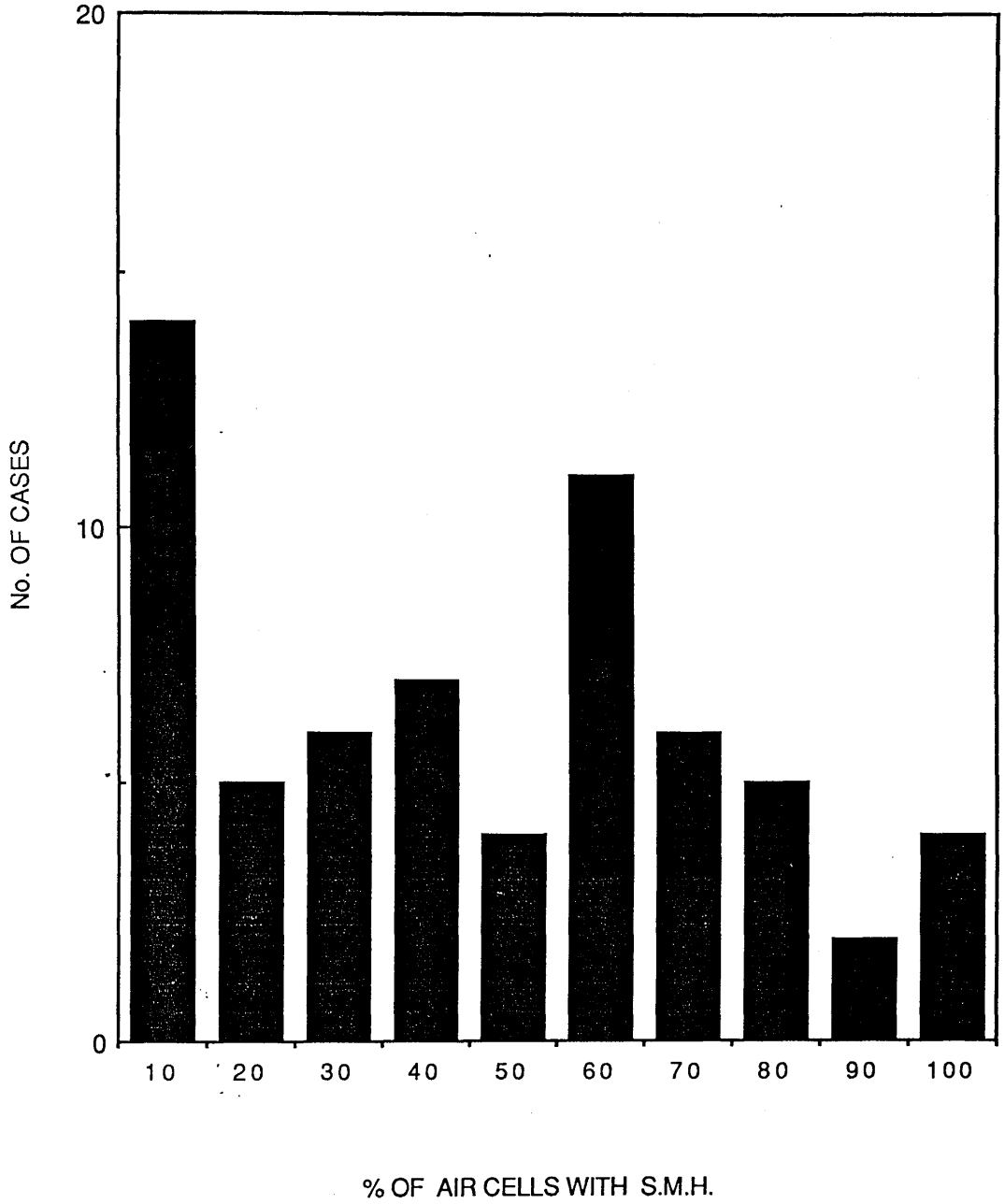

Fig. 8. Description of the percentage of the air cells affected by S.M.H. in drowning cases

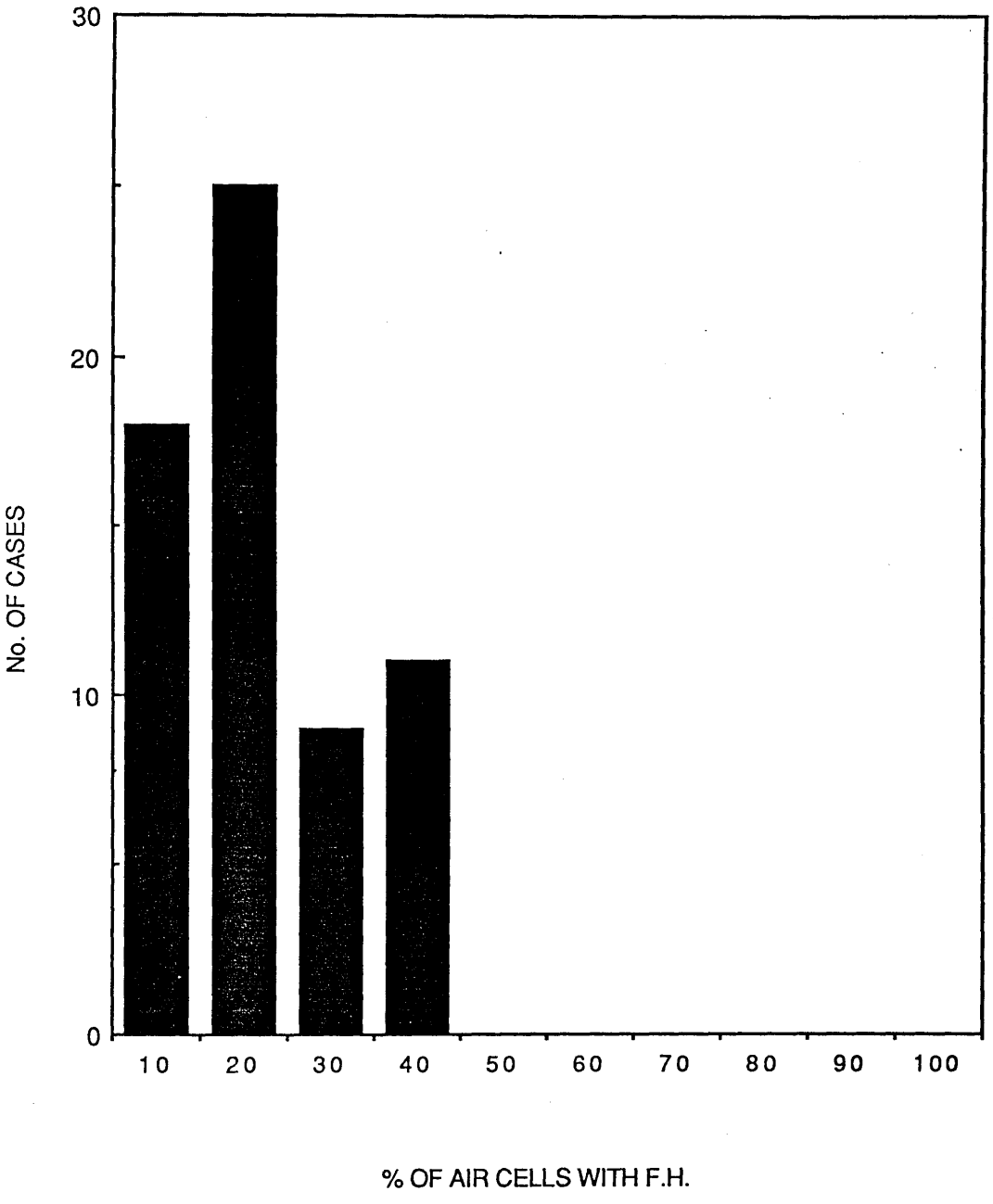

Fig. 9. Description of the percentage of the air cells affected by F.H.in drowning cases.

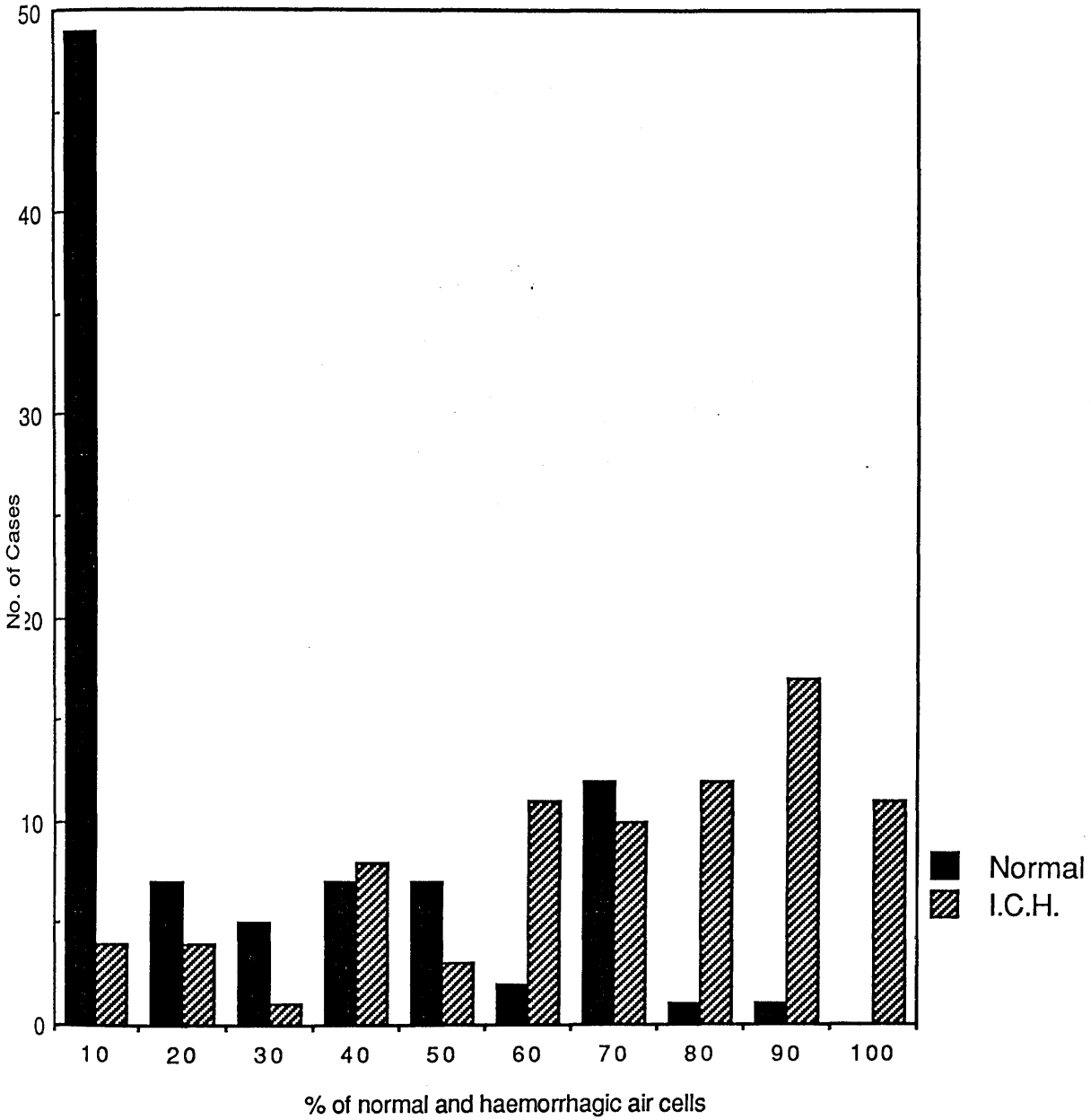

Fig. 10. Comparison between percentage of air cells affected by I.C.H. and normal air cells in drowning cases.

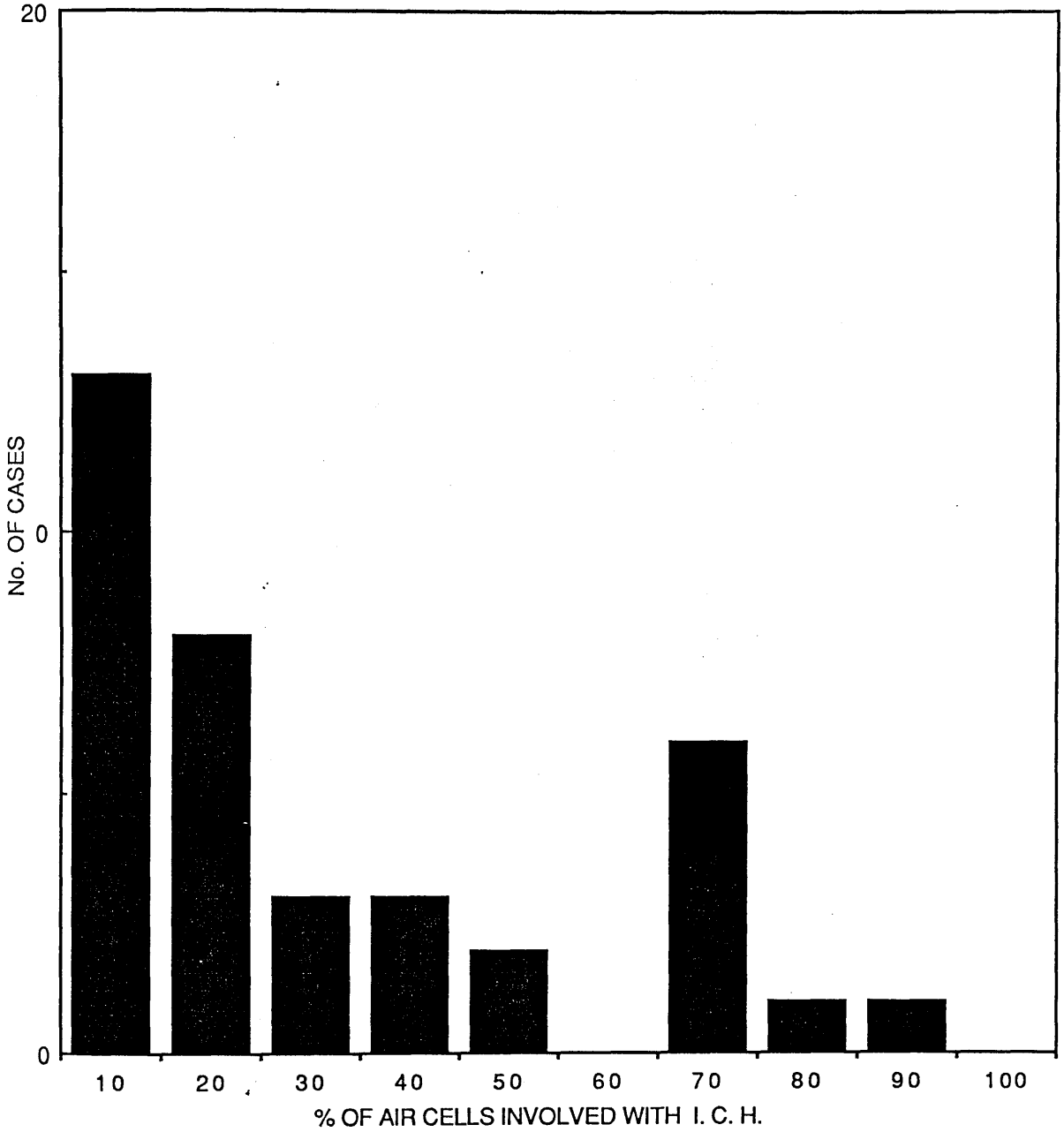

Fig. 11. Description of the percentage of air cells affected by I.C.H. in cases of asphyxia (including hanging cases).

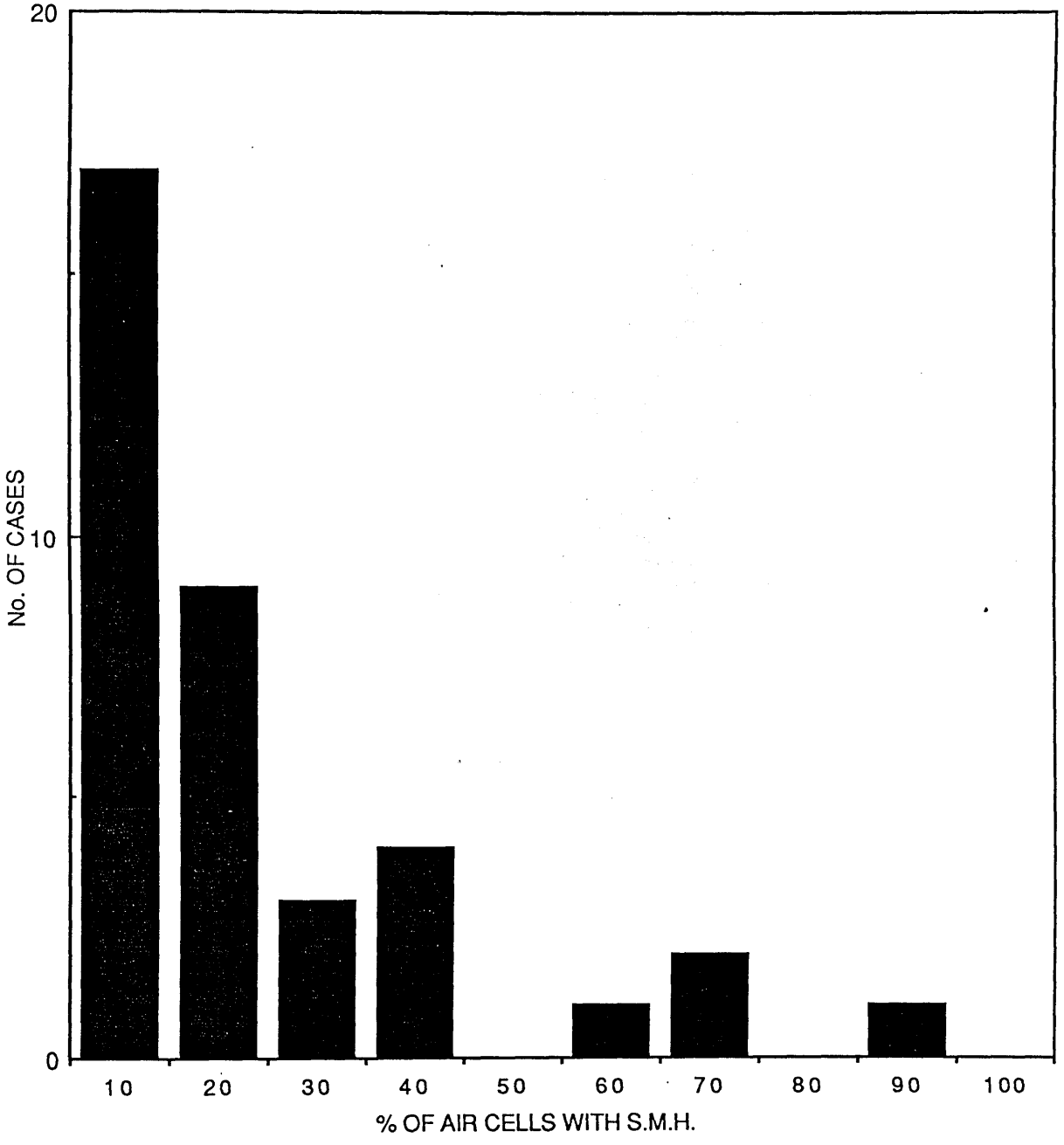

Fig. 12. Description of the percentage of air cells affected by S.M.H. in cases of asphyxia (including hanging cases).

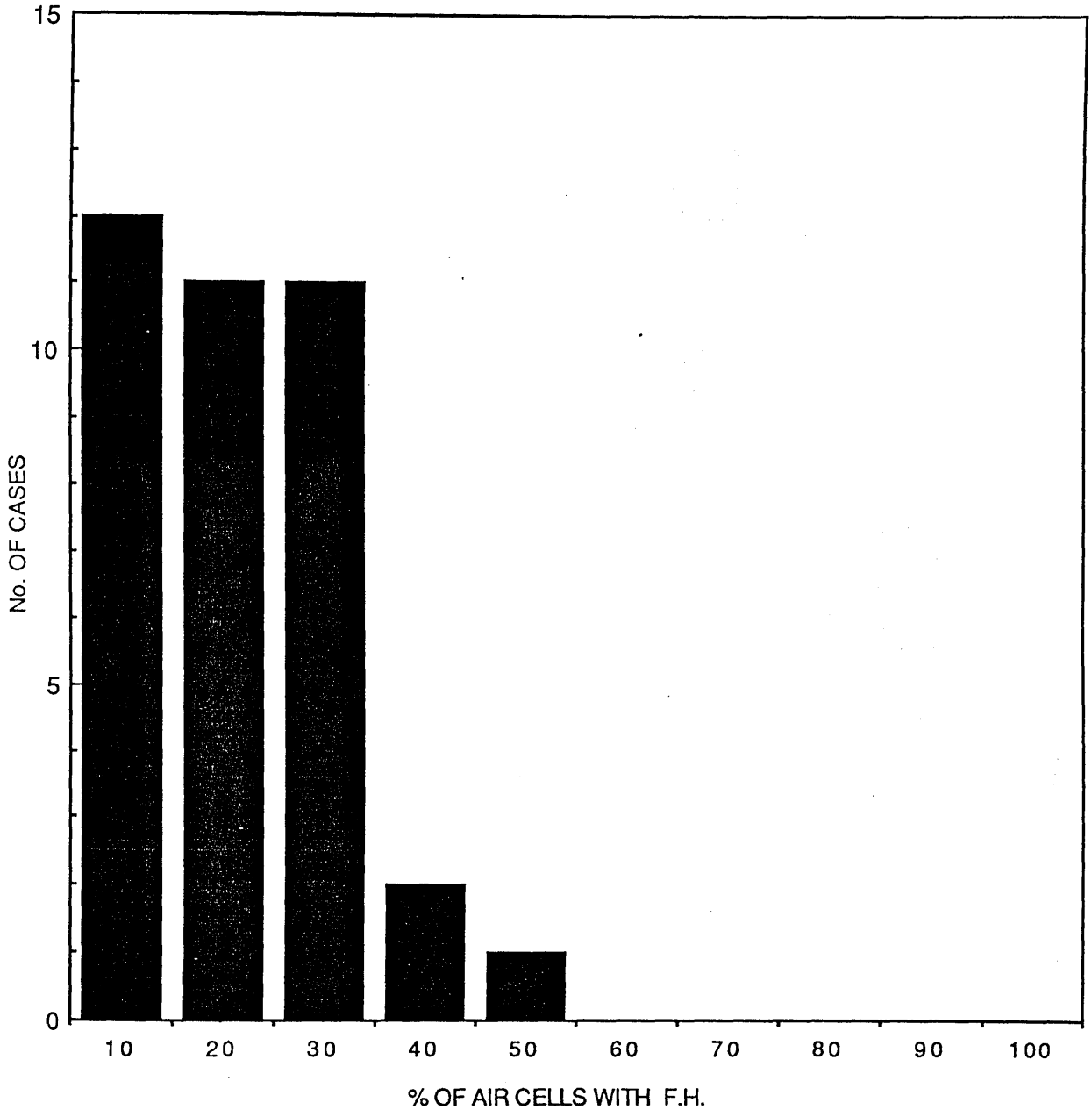

Fig. 13. Description of the percentage of air cells affected by F.H.. in cases of asphyxia (including hanging cases).

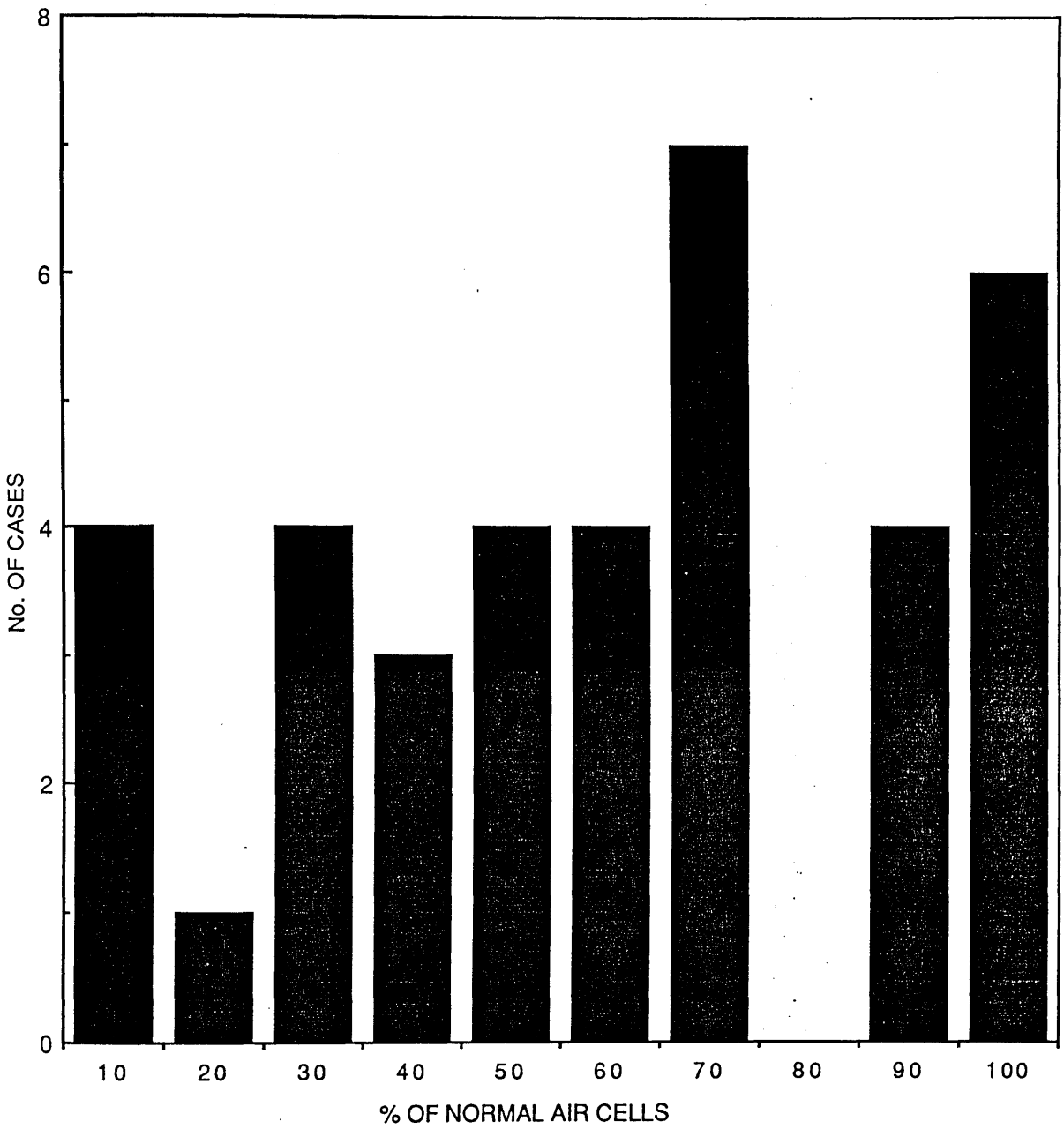

Fig. 14. Description of the percentage of normal air cells in cases of asphyxia (including hanging cases).

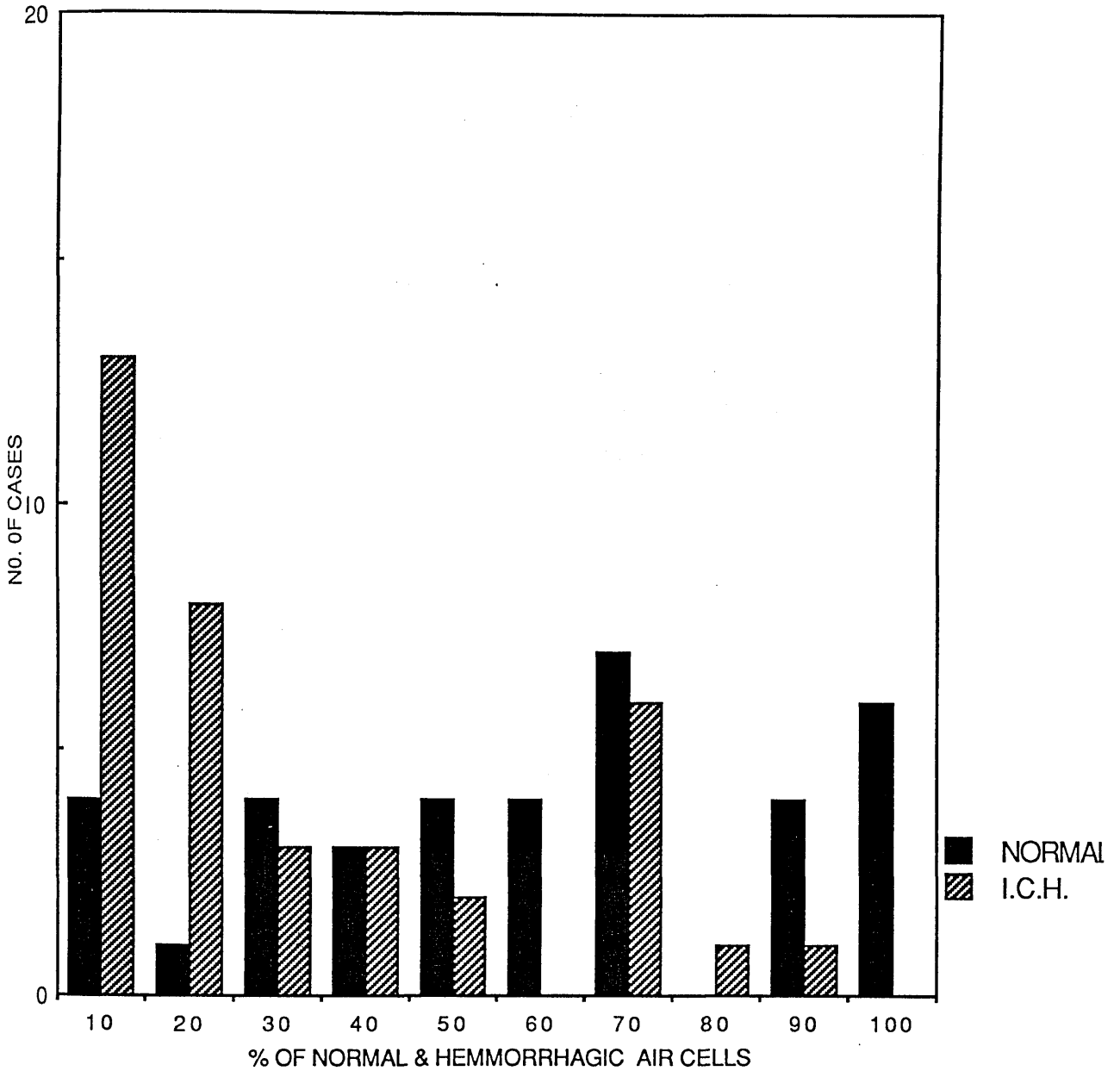

Fig. 15. Comparison between the percentage of air cells affected by I.C.H. and normal air cells in asphyxia (including hanging cases).

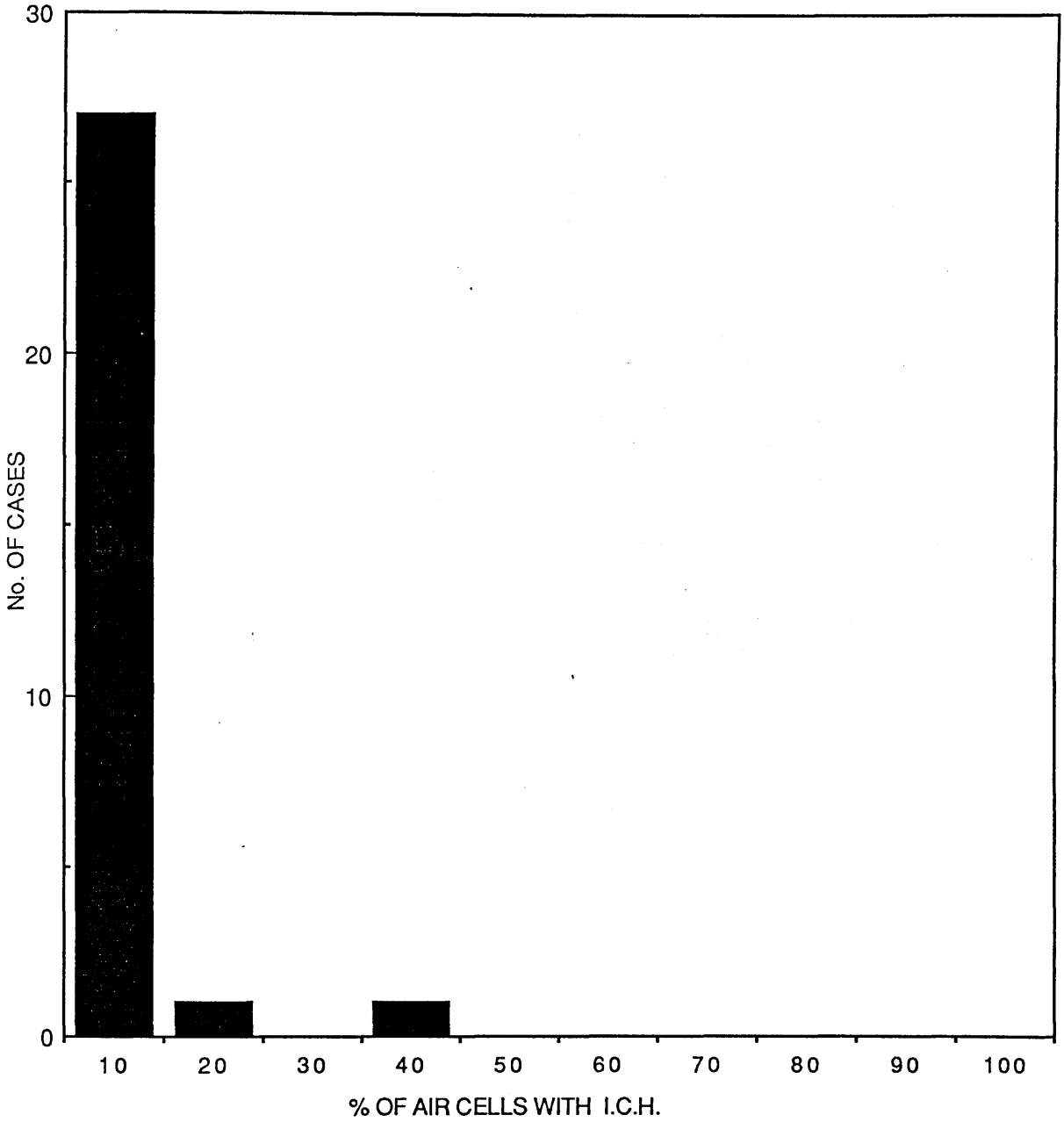

Fig. 16. Description of the percentage of air cells affected by I.C.H. in cases with miscellaneous causes of death (including Ischaemic heart disease).

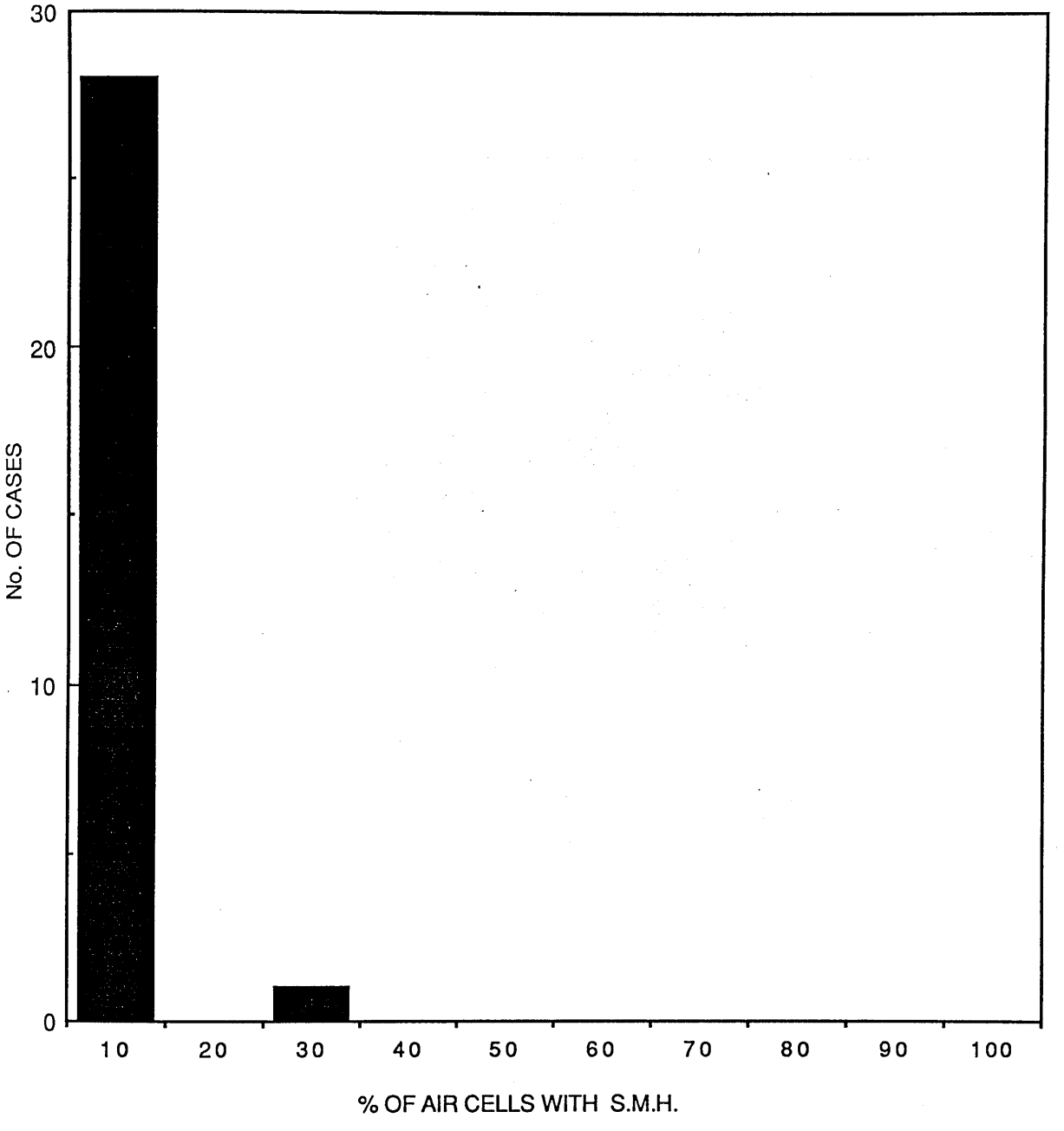

Fig. 17. Description of the percentage of air cells affected by S.M.H. in cases with miscellaneous causes of death (including Ischaemic heart disease).

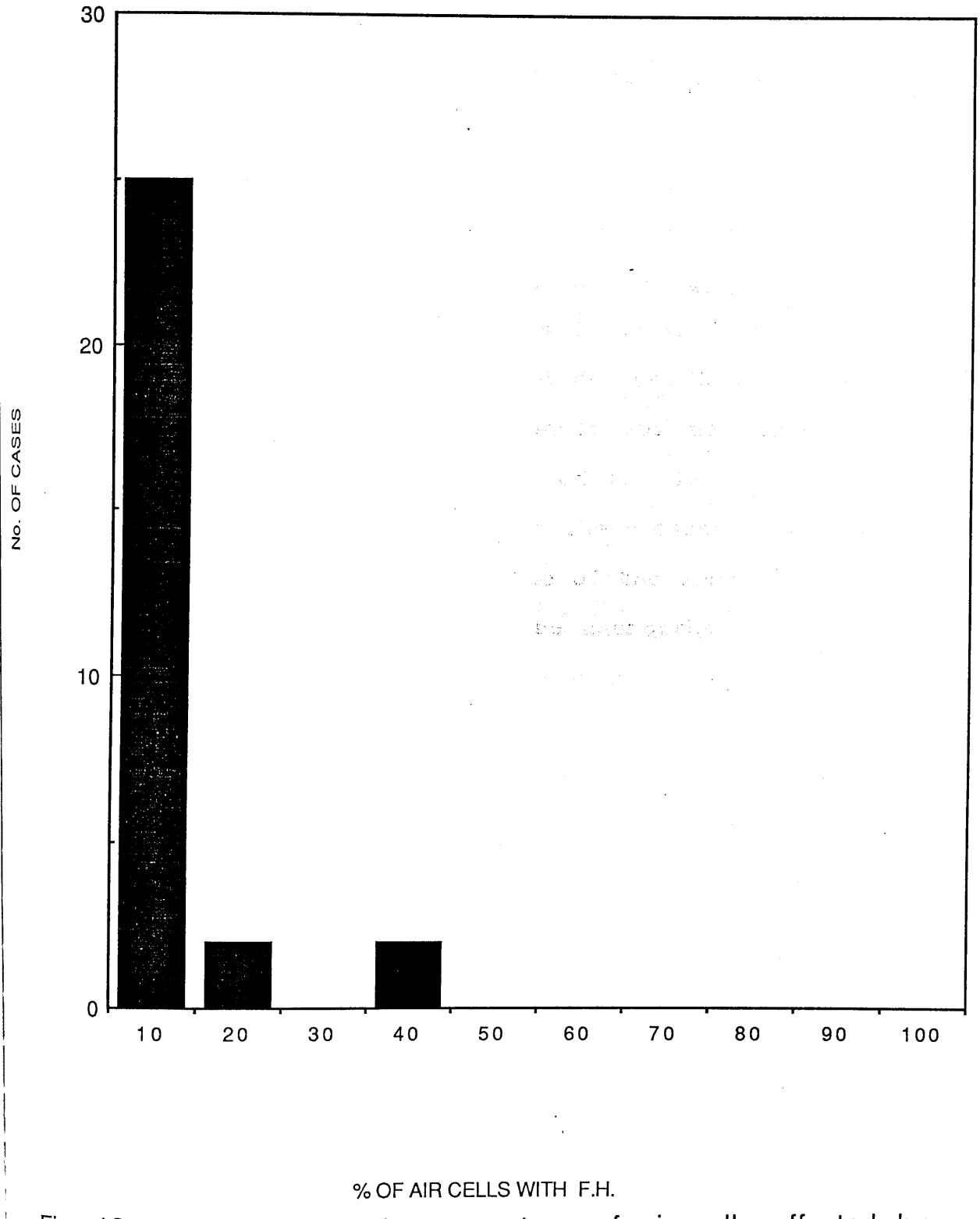

Fig. 18. Description of the percentage of air cells affected by F.H. in cases with miscellaneous causes of death (including Ischaemic heart disease).

Histopathological examination is advantageous in the diagnosis of drowning in cases with advanced degrees of decomposition since it was shown from the results in 18 bodies with these changes. I.C.H. was obvious enough to be diagnosed easily.

The severity of I.C.H. does not seem to be affected by the age, or sex of the drowned victims which were subjected more or less to same circumstances. However, attention was paid to study the possible effects of depth of water on the severity of haemorrhage. In shallow water, e.g. bathtub, I.C.H. was found to involve few air cells and even then in a mild degree. However, it has been claimed that depth of water was not found to be significant in the production of the lesion (122,180), yet it has been emphasized that the pressure differential across the cavity lining is one of the important factors influencing the severity of the haemorrhage. The effect of the depth of water on the severity of the haemorrhage has been accepted to a degree according to the results of bathtub-drownings as shown in Table 5.

In most bathtub drownings, the number of normal air cells would appeared to be higher than the number of air cells involved in any other kind of haemorrhage. This phenomenon of normal air cells could differentiate cases of bathtub and deep water drowning, since it is shown by comparison between normal and intracavitary haemorrhagic air-cells (Fig.10). This phenomenon of I.C.H. is a prominent feature in drowning compared with other conditions such as asphyxia and hanging (Fig.11),

ischaemic heart disease and the miscellaneous group (Fig.16).

Deep water drowning cases with mild degree I.C.H. have shown other pathological changes in the lining mucosa in the form of S.M.H. or F.H. involving most of the air cells.

The physical status of the victim before drowning was raised as a possible factor in explaining the variability in the severity of the lesions. It was concluded that a fit and conscious person might sink and rise to the surface several times in panic conditions, inhaling varying quantities of water which might in turn invade the tympanic cavity. These mechanisms of invasion of the tympano-mastoid spaces with water and the influence of differential pressure while sinking or rising on the cavity lining and reflex laryngospasm forcibly exaggerated in such persons might lead to intensification of the haemorrhage. Such mechanisms presumably happened in those cases who presented severe intrapyramidal haemorrhage whether I.C.H. or S.M.H. or both. However, it does not conform with the results found where victims were weak and drowned quite easily while under effect of alcohol or dying from some other natural disease at the time of their immersion in the water. Some showed a severe degree of I.C.H. in many air cells, which could not be explained by the same mechanism which explained the severity of the lesion in conscious and fit victims. Cases of alcohol intoxication and serious natural disease

seem to drown more rapidly. It has been shown that the sudden cooling of the skin, augmented by the cold receptors may possibly lead to inhalation of water or to cardiovascular collapse. Such reactions may be abnormally strong after consumption of alcohol, which in turn may contribute to the high frequency of drowning while drunk. In a person under the influence of alcohol, there is a strong possibility of death from sudden cardiac arrest at the time of immersion. Reflex cardiac inhibition is thought to be initiated through stimulation of the cold receptors of the skin which are claimed to be exaggerated in cases of alcohol intoxication.

The postmortem presentation of these cases were the dry type drowning in which no indication of the inhalation of water could be demonstrated, and death seemed to happen instantaneously. Such a view has been confirmed by the results obtained from the cases of immersion syndrome which failed to show significant intrapyramidal haemorrhage. This supported the view that death had occurred before any other mechanism had time to produce haemorrhage. Moreover, positive findings of high levels of alcohol in some of the immersion cases justified death by sudden cardiac arrest by sudden immersion in very cold water. Not only a high level of alcohol might precipitate into death by sudden immersion in water, but low levels might contribute to the aetiology of a hypersensitivity reaction. Circumstantial evidence of the sudden death in such cases supports the theory of hypersensitivity

reaction. However, this is unusual as severe degrees of intrapyramidal haemorrhages were detected in cases with high levels of blood alcohol.

The phenomenon by itself is considered to be a vital one and could not have occurred postmortem. Accordingly, it is possible to differentiate between death due to the immersion syndrome and drowning on the basis of middle ear and mastoid air cell haemorrhage. Also, the haemorrhage, being a vital change, helps to decide the presence or absence of life at the time of immersion. Such a conclusion can be reached by the exclusion of a few easily diagnosable conditions that may cause the same reaction, e.g. intracranial haemorrhage, carbon monoxide poisoning and hanging. On the other hand, temporal bone examination revealed possible mechanisms of death of 4 bodies recovered from water and attributed to causes other than drowning or immersion. Two of them showed a mild degree of I.C.H. with involvement of less than 12% of the air cells in one; while a second showed more than 50% of the air cells to be affected. These results confirmed the possibility of inhalation of water or that the victims were subjected to the effect of pressure differential. The possibility of inhalation of water was confirmed by the finding of a moderate amount of water and a relatively large stone in the stomach of the first case who had been rescued alive, but died a few minutes later. One of the 4 cases was excluded as a drowning death by the absence of any haemorrhage in the air cells. The fourth case was a young man rescued from a swimming

pool in a semi-conscious condition. Necropsy revealed pulmonary haemorrhage and oedema with aspiration of the stomach content as the cause of death. However, temporal bone examination revealed that more than 50% of the air cells contained a moderate amount of I.C.H.

Our view, concerning these cases favours drowning as being the cause of the intrapyramidal haemorrhage.

Generally, comparing the findings of those in drowning with those in hanging, the most distinctive histological feature was the engorgement of the blood vessels, especially those within the lamina propria of the air cells. Oedema was variable but generally it was milder than that found in definite drowning cases.

In most hanging cases, a few air cells were detected to contain only mild haemorrhage. Failure to find any I.C.H. in 2 cases encourage the view that possibility of reflex vagal inhibition of the heart was the cause of death rather than asphyxia. Absence of a severe degree of I.C.H. and S.M.H. in most cases of hanging does not justify the high venous pressure and congestion as the cause of severe haemorrhage. Another possible mechanism of severe haemorrhage is an increase in the permeability of the walls of the blood vessels as a result of anoxia. This seems to be an exception rather than a rule, as only two cases of pure anoxia, i.e. cases of carbon monoxide poisoning, showed a severe degree of I.C.H.

4.2.3. Submucosal and Focal haemorrhage :

Other important features in drowning include the detection of red blood corpuscles within the lining mucosa of the middle ear and mastoid air cells (Fig.8). Two types of haemorrhage are distinguished, S.M.H. and F.H. The severity in degree or grade is variable, and like I.C.H. no single factor could explain such variability. The percentage of air cells that showed S.M.H. was found to be significantly high and closely similar to the number affected by I.C.H. in typical drowning.

We found, for example, that 75% and 8.7% of air spaces in a drowned boy displayed S.M.H. and I.C.H. respectively. This victim, a teenage male, was witnessed to have drowned in a rapidly flowing burn. He was discovered later to have gross haemorrhage of the outer surface of both temporal bones (Case No 1) Table (4). Hence the diagnosis of drowning can be exclusively based on the finding of S.M.H. alone.

The high number of air cells affected by moderate to severe degrees of S.M.H., was rarely found in death other than drowning (Figs.12 and 17), unlike F.H. which being very mild in more than 50% of cases (Fig.9). By contrast F.H. was found to be the main pathological sign in the tympano-mastoid area of 17 cases of hanging (Tab 18) or in the 27 cases of asphyxia and hanging together (Fig.13). This confirms the view that the engorgement of blood vessels and increased venous pressure are the main

factors of the lesion in cases of obstruction to the venous return like in hanging. Such a mechanism is unlikely to be the cause of haemorrhage in drowning. This view is based on the grounds that S.M.H. in hanging cases and F.H. in drowning cases are contradictory findings to be explained on a single basis i.e. congestion of blood vessels.

4.3. MECHANISM OF HAEMORRHAGE :

Increased venous pressure due to a violent respiratory effort against a closed glottis in laryngospasm, which occurs during the process of drowning may lead to transudation of serum into the lamina propria with eventual rupture of the blood vessels. This mechanism could not explain convincingly the intrapyramidal haemorrhage in drowning. However there are other possible mechanisms which might explain the phenomenon. One of these is water invasion of tympanic cavity via the Eustachian tube(102,115,180). Such invasion has been experimentally confirmed and microscopically demonstrated by finding of amniotic fluid containing desqumated squames cells in the middle ear of asphyxiated infant (19). Such an invasion of the typmanic cavity by water caused anoxia and increased the negative pressure in air cells which might have aggravated the fragility of the poorly supported blood vessels within the oedematous lamina propria with eventual rupture of these blood vessels. The irritant effect of aspirated fluid on the vascular mucosa may contribute to rupture of the vascular

wall. The theory of invasion of tympanic cavity by water is supported by the negative findings of haemorrhage in the cases of the immersion syndromes which failed to show the classical signs of inhalation of water.

Anoxia appears to have an essential role in pathogenesis of the haemorrhage in drowning. The view is endorsed by the findings presented in pure anoxia, e.g. carbon monoxide poisoning. Experimentally, persistent hypoxia was incriminated as the main physiological change in both fresh and salt water drowning (56,112,113,114,163). A second and more acceptable mechanism is thought to be similar to that of barotrauma (7,102,115,122), from observations of middle ear haemorrhage as a recognised result of under-water swimming (45,107). Barotrauma is a well-known sequel of diving into as little as 6 feet of water (99). It defines as tissue damage resulting from the change in the volume of gas spaces due to change in the ambient pressure which being the force applied per unit area. The units of pressure commonly used are atmospheres (atm), pounds per square inch (psi) and millimeter of mercury (mm Hg). For instance, at sea level the atmospheric pressure will be 14.7 psi or 760 mm Hg, while the absolute pressure is the pressure of water plus the atmospheric pressure. Accordingly the pressure exerted on the 3 main compartments of the temporal bone (i.e. external, middle and inner ears) will be similar at the sea level. But during deep descent into the water, there will be

compression of the air explained by Boyle's law in which a volume of a gas varies inversely to the pressure and vice versa. Consequently, the pressure differential between a closed cavity and the surrounding environment at a depth of 2.6 ft. might be 60 mm Hg, 90 mm Hg at 3.9 ft. and 100-500 mmHg at depth of 4.3-17.4 ft. As a result of the pressure differential between the middle ear and the surrounding water the formation of a relative vacuum will be created (45) with swelling of the soft tissue lining of these cavities by virtue of its ability to accept fluid from the rest of the body, or by negative pressure created. This leads ultimately to the rupture of the poorly supported vessels of lining mucosa. For that reason, the theory of pressure differential is presumed to be responsible for the variation in the severity of the haemorrhage (115). It is obviously of doubtful validity in some cases of shallow water drowning. The acceptable theory in bathtub drowning, is the anoxia created by water in the upper air way leading to increased resorption of air and increased negative pressure. This induces oedema and vascular congestion making the middle ear and mastoid air cells more sensitive than normal to barotrauma.

In terms of its effect on a swimmer, the haemorrhage is thought to be a significant cause of drowning by interfering with inner ear function and inducing vertigo, with loss of orientation of the swimmer. Vertigo occurs without morphological changes in the inner ear. It is

either caused by abnormal pressure alone or in combination with intrapyramidal haemorrhage. It is commonly experienced by divers.

Generally our findings confirm those of other workers (93,115,122). The lesion varies from submucosal haemorrhage with congestion to massive bleeding filling most of air spaces. It is an highly characteristic sign and is supportive evidence for a diagnosis of death by drowning.

5. CONCLUSION :

The study of 85 typical drownings, 8 possible drownings, 17 hangings, 15 cases of ischaemic heart disease, 10 death from asphyxia, 5 of head injury, 5 carbon monoxide poisoning and miscellaneous causes of death, was conducted to determine the reliability of intrapyramidal haemorrhage as a distinctive sign of drowning and assessing the reliability of microscopical and naked-eye examination of the temporal bone.

The following results emerge :

1. Naked-eye examination of the outer surface of the temporal bone is often helpful in detecting haemorrhage within the middle ear and mastoid ear cells. Usually it is brownish-red discoloration just beneath the semi-translucent tegmen tympani.

2. Bilateral microscopical examination at 5-6 different levels of the bone are sufficient for the investigation of intrapyramidal haemorrhage.

3. Intrapyramidal haemorrhage presenting as

intracavitary bleeding was highly characteristic in 79 out of 81 well penumatized right temporal bones and in 52 out of 55 left bones.

4. It was a positive sign bilaterally in 52 out of 55 cases of drowning.

5. Submucosal haemorrhage is, also, an important sign and is considered a significant pathological feature in the diagnosis of drowning.

6. I.C.H. and S.M.H. are not pathognomonic signs of drowning, but are common findings to be considered as characteristic criteria of drowning when other causes are excluded.

7. Intrapyramidal haemorrhage was detected in cases other than drowning e.g.in hanging, carbon-monoxide poisoning, subarachnoid haemorrhage, skull fracture and chlormethiazol poisoning.

8. Increased venous pressure, anoxia, invasion of tympanic cavity by water and barotrauma, acting singly or collectively, are considered as causative mechanisms of the haemorrhage.

9. The diagnosis of immersion syndrome, often difficult to reach, may be established by not finding haemorrhage in the tympanomastoid region.

10. Histological examination of the tympanomastoid area may establish or exclude a diagnosis of drowning even in the absence of the classical signs of inhalation of water or presence of other natural causes.

11. The lesion is a vital phenomenon; by excluding

other conditions, it can establish whether the victim was alive or dead before immersion in water.

12. The lesion is considered a sequel of drowning as well as one of its causes.

CHAPTER 4

MAGNESIUM, CALCIUM, POTASSIUM, SODIUM AND CHLORIDE IN POST-MORTEM VITREOUS HUMOUR FROM HUMANS, REGARDING THE ESTIMATION OF IMMERSION TIME IN FRESH WATER DROWNING VICTIMS

1. INTRODUCTION AND LITERATURE REVIEW :

The question of the time of death has been repeatedly encountered by forensic pathologists and medico-legal practitioners (5,18). The estimation of time of death is one of the most important and fundamental problems in forensic medicine.

The significance of post-mortem interval concentrates on its decisive value to the outcome of a case of suspicious death particularly in relation to homicide (79,94,95,103,123). It is also of great importance in some civil proceeding (94,95,103). However the so called moment of death has an investigational rather than evidential value (5,95,105). This is because of the lack of an accurate, reliable, and easily applicable method of estimating the time since death (95,103,105,132). The other reason is the fact that the time of death does not necessarily represent the time of assault (103,105). However, there are many histological and histochemical methods which have been used to estimate the age of wounds and other injures(52).

Apart from entomological methods which have been used

for the estimation of post-mortem interval (6,132), most studies have been based on physical changes of the body after death (50,65,132). These changes include rigor mortis, livor mortis, algor mortis, ocular changes, putrefaction, adipocere formation and other histological changes. The distribution and extent of rigor mortis do not give more than an inaccurate indication. This is because the order and velocity of the spreading of rigidity as well as its disappearance, are by no means as regular as might be expected (24,40,141). The biochemical changes after death, in which ATP-ADP shift plays a major role in the onset of muscular rigidity, have been investigated but have been unsuccessful in estimating the time of death (31, 98).

Similarly the observation of post-mortem lividity gives no more accurate information about the time of death (123). The onset and the degree of lividity seem to be variably influenced by the antemortem physical conditions of the deceased, the cause of death and the environmental conditions(24). The colour of the livor may be used to ascertain the cause of death but give no further information concerning the moment of death (21).

Algor mortis or post-mortem cooling has been considered as an indication of the post-mortem interval by measuring the loss of heat after death. The hope of finding a simple measurement of body temperature as a reliable and easy method of determining the post-mortem interval has never been completely fulfilled (40,106,144). One of the

reasons is that there is no such thing as regular fall of temperature after death (141). Also the cooling rate greatly depends on the environmental conditions (141). Furthermore the site at which the temperature is taken plays an important part (141). Nevertheless the measurements taken are often multiple and the large number of variables(e.g. physical development, age, sex, cause of death, clothing, environmental temperature, movement of surrounding air) are often difficult if not impossible to assess (123). Although regarded as the most reliable method in the practice of forensic medicine, its value is still limited to the first 18-24 hours postmortem (5,103,105,132).

Intra-ocular pressure, as one of the means for estimation of the time of death, does not seem very reliable (123). Moreover the method is only applicable for a short time after death.

Retinal examination was held to be a promising tool for the estimation of time since death by correlating different retinal features with the time after death (86). However, there are many variables which make the application of this method unreliable (185).

The excitability of the small muscles of the eye, mouth and hand by means of electric stimuli has been measured (141), for use in correlation with the time since death. The disadvantage of being limited to a very short time and the dependence on the muscular glycogen concentration, render the method, like other physical changes, highly unreliable.

Generally the use of the physical signs of rigor mortis, lividity, algor mortis and decomposition are widely described but give rather imprecise estimates because they are affected by so many variables (77).

As the estimation of the postmortem interval using the above methods has proved inconclusive, several workers have tried to find the relationship between the post-mortem biochemical changes in various body fluids and the time since death.

Body fluids such as blood, cerebro-spinal fluid, synovial fluid and pericardial fluid have been analyzed to find the most reliable postmortem chemical change which can be used as a postmortem clock (2,3,4,13,14,28,29). Examples of substances which have been included in these studies are carbohydrates, (glucose, inositol, lactic acid, pyruvic acid and ascorbic acid) electrolytes (sodium, potassium, calcium, phosphorus, sulphide, magnesium chloride, iodide and selenium) and other organic compounds (cholesterol, bilirubin, urobilinogen, protein, amino acids fatty acids and vitamin E). Postmortem changes in the serum begin almost immediately after death, before haemolysis or histological evidence of tissue deterioration set in (77). Because of rapidly progressing bacterial contamination, the strict dependence on environmental factors and the different biochemical parameters during life, blood and serum as substrates for examination have proved unsuitable (123,141). The individual rate of

change is so variable from one case to another that no precise estimate of the time of death can be made from any single determination. Cerebrospinal fluid has been investigated as a possible source of a cleaner body fluid for studying postmortem chemical changes, since it is more isolated from decomposing tissue than serum and since it is virtually acellular in the absence of intracerebral hemorrhage. However the investigations showed that biochemical changes occur rather quickly but erratically (27) after death and can be misleading. The collection of the fluid uncontaminated by blood or other materials can be difficult (28,118). These chemical changes offer little real advantage over the classical methods (101).

In more recent years, analysis of chemical changes in the intraocular fluid after death has come to the fore as an additional way of estimating the time of death (2,27,74).

The eyeball is isolated and well protected anatomically. As a consequence vitreous humour is usually preserved despite serious trauma to the head and is much less subject to contamination or putrefactive changes than either blood or cerebrospinal fluid (27).

The measurement of the composition of human intraocular fluid in enucleated specimens following trauma was performed many years ago (160), and animal work has paved the way for major breakthroughs in the field of ocular biochemistry (36). However, virtually no information is

available on the normal antemortem composition of human intraocular fluid. Presumed values must be obtained from the extrapolation of postmortem values. The most important property of vitreous humour, in contrast to the blood and cerebrospinal fluid, is that the chemical changes for many substances occur more slowly and are therefore more easily detectable in the vitreous humour than any other body fluid (27).

Biochemical analysis of vitreous humour has been used in the diagnosis of many conditions such as dehydration, the hyponatraemic state, diabetic ketoacidosis, uraemia, and various toxicological conditions (27). The most notable examples have been the attempts to use vitreous humour chemical data in the investigation of sudden infant death syndrome (12,157,159) and in the estimation of postmortem interval by means of the time-related increase in potassium concentration (2,3,27,83,101,160).

1.1 ESTIMATION OF IMMERSION TIME :

The estimation of the period of submersion has been made by evaluating the degree of postmortem change (61,84). These changes include maceration of the skin, cooling of the body, rigor mortis, hypostasis, decomposition and adipocere formation. However, most of studies have concentrated on the correlation between the biochemical changes in the biological fluids, especially the vitreous humour, and the time of immersion (4,15,51,158).

1.1.1. HISTOPATHOLOGICAL METHODS FOR ESTIMATING THE IMMERSION TIME :

Moderate retention of stainability and distinct creasing of the skin without epidermal detachment indicate up to 2-3 weeks as a probable period of immersion. However, systematic histological investigation of the wrinkling does not reveal a regular connection between the time in the water and time of appearance of morphological changes in the epidermis (84).

Histological examination of lung tissue has been used to estimate a period of immersion in water of not less than 2 months. The elastic fibers of pulmonary tissue do not show distinct reduction in stainability and structure until the corpse has been in the water for over 2 months (84). Well preserved pulmonary elastica does indicate a period in the water of less than 2 months . Beyond this time the very diverse findings cannot be systematically presented.

There are other histological criteria which indicate immersion in water, such as lime soap nodules found on the intima of veins in particular the hepatic veins and the endocardium (84). Nodules composed of calcium phosphates, calcium carbonate, neutral fat and proteinaceous substances may be demonstrated in the skin of immersed bodies (84). However, the presence of these signs do not relate to the immersion time and merely confirm a long time of immersion has elapsed. In the cases of sea water drowning, long immersion can be

established by the finding of struvite crystal in the skin (84). Finding larger crystals may indicate immersion times of over 9 months.

Adipocere is a soft, whitish and sometime greasy material which consists mainly of fatty acids created by the postmortem hydrolysis and hydrogenation of body fat, mixed with the remains of muscles, fibrous and nervous tissue and small amounts of soap (104). This material is formed within 5-6 months in cool climates, 5-6 weeks at the height of the European summer and 3 weeks in countries like Egypt and India (61). The formation of adipocere is influenced by many factors such as, temperature, humidity and putrefaction. The condition may also occur outside water in damp and cool environments where air is excluded (84).

Immersion time has also been estimated by bacteriological examination of the skin with the finding of chromogenic bacteria in the cutis of the body after 1-2 weeks, though this is variable (84).

Entomological examination may provide supporting evidence in estimates of the immersion time. The fleas on a body usually drown in 24-27 hours. If they have been immersed for a period of between a few hours and 12 hours, they can be revived in from 20 minutes to 1 hour respectively. If they have been immersed for 24 hours, they only partially recover within 4 hours (148). However, the method rarely has any practical value (61).

1.1.2. BIOCHEMICAL METHODS OF ESTIMATING IMMERSION TIME:

Several studies have been carried out to estimate the time of immersion in water by examination of chemical changes in the biological fluids, incluiding the intraocular fluid.

Most studies have focused on serum electrolyte changes in drowning and near-drowning and only a few workers have examined the vitreous humour chemistry (4,15,16,51,158).

Adjutantis and Coutselinis (4) studied the effects of sea water submersion on exenterated human eyeballs, and demonstrated the movement of magnesium ions into the vitreous humour They concluded that the rate of diffusion of magnesium ions into the eye is proportional to the time elapsing after death and that the concentration in the vitreous humour might be used to determine the time that the cadaver has been immersed in sea water. Sturner et. al. (158) used bovine eyeballs in a similar, but more extensive study. They found that the vitreous potassium concentration plateaued after 16 hours in sea water .

In an other study, vitreous humour analysis was performed on 13 air crash victims who had remained immersed in near-freezing fresh water for seven or eight days (16). Evidence of the dilution of the vitreous humour electrolytes was noted. This supports the idea that the vitreous humour potassium concentration shows the least individual variation of the chemical determinations used to estimated the postmortem interval.

Bray (15) studied postmortem vitreous humour chemistry

in 25 cases in which death was followed by submersion in fresh water for known lengths of time. He found that by employing the weighted average of the potassium and chloride concentration the duration of submersion in cold water could be determined over the range of 2-10 days.

Farmer at. el. (51) have demonstrated post-immersion diffusion of electrolytes across the permeable membrane of the eyeball. Magnesium in salt water cases, and sodium in fresh water cases, are related, albeit erratically, to the period of the immersion.

In this work, post-mortem vitreous humour studies were carried out to determine whether vitreous humour electrolyte concentrations might reveal a distinctive pattern related to the circumstances of death, the postmortem interval, or to prolonged immersion in fresh water. The possible use of routine electrolyte studies for estimating immersion time was the principal objective of this study.

1.3. ADVANTAGES OF USING VITREOUS HUMOUR :

The eyeball is isolated and well protected anatomically. As a consequence the vitreous humour is usually preserved. Also vitreous humour is less subject to contamination or putrefactive changes than either blood or cerebrospinal fluid. Therefore vitreous humour is considered the best fluid for postmortem chemical analysis.

With care, approximately 2 ml. of clear crystal fluid

can be obtained from each eye by using a syringe with a No.20 needle inserted into the center of the eyeball through the outer canthus of the eye. This amount provides sufficient material for the determination of Mg, Ca, K, Na and Cl.

Such clear specimen of vitreous humour may even be available in bodies showing early decomposition, in contrast to blood specimens where extensive hemolysis of red blood cells is common.

The vitreous humour once obtained may be refrigerated for a lengthy period without deterioration.

This technique of aspiration of vitreous humour leaves no visible marks on the body, and the shape of the eyeball is restored by injecting tap water into the eye.

The important advantage of using vitreous humour is that the chemical changes occur much more slowly than in cerebrospinal fluid or blood, enabling in some cases detection of antemortem abnormalities.

Intraocular fluid consists of the aqueous fluid which fills the anterior and part of the posterior chamber and vitreous fluid which permeates the vitreous body. The chemical composition of vitreous humour is complex in that it constitutes an ultra-filtrate of plasma with regard to some of its constituents but not with regard to others (36). The chemical difference between aqueous and vitreous fluid are, however, minor and of no significance for the purpose of this study. But, because the vitreous humour is more protected from the environment and has a

greater volume than the aqueous, it is preferred for the analysis of intraocular electrolytes.

In addition vitreous humour is preferred to blood especially in cases of drowning, for the investigation of postmortem interval and immersion time. Since the submersion exposes primarily the aqueous compartment of the eye as the foremost liquid barrier, rather than the vitreous, the two being separated by the lens and ciliary process.

1.3. DISADVANTAGES AND PRECAUTIONS IN USING VITREOUS HUMOUR :

Virtually no information is available on the normal antemortem composition of human intraocular fluid. The few series which have been collected have used surgically removed eyes as the source of vitreous humour, and these must be considered suspect in the light of the occasional striking variations shown in the chemical composition of diseased eyes studied postmortem (77).

The fluid used for analysis should be crystal clear, otherwise contamination of vitreous humour with cells or blood renders it useless for analysis.

An other point when using vitreous humour is to avoid aspiration of less than 0.5 ml. because such a low volume may yield unrepresentative results, owing to the uneven distribution of some metals like potassium within the vitreous body (101).

Forceful aspiration of vitreous humour should also be avoided, as it may cause detachment of retinal cells subsequently leading to erratic results.

2. MATERIAL AND METHODS :

The cases forming this study were composed of control and drowning cases. From October 1985 to December 1987 the vitreous humour was collected at Necropsy by needle aspiration from the posterior chamber of both eyes. The major causes of death in the 48 control cases were heart disease (18), hanging (9) and intracranial haemorrhage.

The controls comprised 25 males and 23 females, aged between 21 and 85 years, with a post-mortem interval between the time of death and sampling of from 8 to 115 hrs. The post-mortem intervals of these cases were estimated from the information obtained from the police or witnesses.

In fresh water drownings the sex distribution was 21 males and 20 females, with a range of age from 4 to 83 years. The post-mortem interval in these cases ranged from 2 h. to 150 h. The important point in cases of drowning and of special importance in the estimation of the time elapsed since death was the immersion time, which is defined as the time elapse between the moment of submersion to the time of recovery of the body from the water. It ranged from 0.20 h to 130 h. The most frequent sites of drowning in these cases were rivers, canals, burns, lochs and bathtubs (Tab.2).

2.1 PREPARATION OF MATERIALS FOR ANALYSIS :

In order to analyse the samples of vitreous humour, the

following procedures of preparation of the samples were followed :

A. The solutions used for preparation of samples (Dilutents).

1. 0.75% Disodium Ethylene Diamine Tetra-Acetic acid (Na_2EDTA), by dissolving 15 g. of Na_2EDTA in 2 liters of de-ionized water.

2. De-ionized water, purified by a Millipore system of high efficiency.

2.2. METHODS OF DILUTION :

1. For magnesium analysis, sample of 100 µl of vitreous humour were made up to 10 ml. with 0.75% Na_2EDTA, i.e. equivalent to a 100 fold dilution.

2. For analysis of calcium, also, 100 µl. of vitreous humour sample were made up to 10 ml. with 0.75% Na_2EDTA, i.e. equivalent to 100 fold dilution.

3. For analysis of potassium, 100 µl. of the sample were made up to 25 ml. with de-ionized water, i.e. equivalent to 250 fold dilution.

4. For analysis of sodium, 100 µl. of the sample were made up to 100 ml. with de-ionized water, i.e. equivalent to 1000 fold dilution.

5. Analysis of the samples of vitreous humour for chloride did not require dilution, but employed a Chloride-meter instrument in which samples of vitreous humour were analysed undiluted.

In order to avoid any metallic contamination of the

glassware used in the process of analysis, thorough cleansing of the glassware was mandatory throughout the course of this work. After use the glassware was cleaned and washed carefully with distilled water, before being immersed in an acid bath for at least 1-2 days. The glassware was taken out of the acid bath cleaned two to three times with purified, i.e. de-ionized waterand dried at the room temperature.

2.3. REFERENCE MATERIALS :

The analytical procedures were validated by multiple analysis of Seronorm (a reference serum for control of clinical chemical analysis supplied by Nyegaard and Co., Oslo, Norway) Batch No. 144. Seronorm was prepared for analysis in the same manner as vitreous humour, i.e. for each 100 µl. of Seronorm, 10 ml. of 0.75% of Na_2EDTA was used for the analysis of Mg, and Ca, 100 ml. of purified water for analysis of Na, and 25 ml. of the same water for analysis of K. The following results (mg/l) were obtained, (Mg) 22.6 ± 1.4; (Ca) 106 ± 2; (K) 189 ± 9; (Na) 3240 ± 100. The certified values for these elements in the Seronorm are 22.4, 109, 189, and 3080 respectively.

2.4. STANDARD SOLUTIONS :

A range of standard solutions for each element was used to construct calibration curves necessary for the calculation of the concentrations in the vitreous humour. Working standard were prepared from stock solutions of

1000 mg/l solutions, supplied by BDH Chemical Ltd, Poole, England.

Analyses were carried out in the air-acetylene flame of a Perkin-Elmer PE 306 atomic absorption spectrophotometer at wavelenght settings of 285.2 nm (Mg), 422.7 nm (Ca), 766.5 nm (K), and 589 nm (Na).

3. RESULTS :

3.1. CONTROL DATA :

The individual Mg, Ca, K, Na and Cl results for controls are plotted in groups corresponding to successive 24 hours postmortem interval (Fig.1A-E). Table(3) shows the range, mean values of Mg,K,Ca,Na,Cl, postmortem interval and age for controls.

The relationships between the individual electrolyte values and the time elapsed since death are depicted in Figs.2-6. Also, the average values of Mg,K, Ca,Na and Cl levels were calculated and plotted against the mean postmortem interval for each 24 hour period (Figs. 7-11).

From linear regression, the nominal electrolyte concentration (mg/l.) at PMI = 0 are 20.9 (Mg), 58.8 (Ca), 3058 (Na), 326 (K), and 4316 (Cl) (Figs.2-6).

The gradient of increase or decrease in concentration of an ion per hour (mg/l.per.h.) are 0.058 (Mg), 0.041 (Ca), -2.05 (Na), -4.97 (Cl) and 5.01 (K).

For the estimation of postmortem interval, by using individual values of vitreous electrolyte concentration, the following regression equations were obtained, in

TABLE 1

ELECTROLYTE CONCENTRATIONS IN THE VITREOUS HUMOUR OF CONTROL CASES

Case No.	PMI (hr)	Mg (mg/l)	Ca (mg/l)	K (mg/l)	Na (mg/l)	Cl (mg/l)
1	8	23.8	73	430	2800	4190
2	15	21.3	65	417	3040	4440
3	18	22.8	62.5	382	2930	4140
4	24	22.9	66	571	3085	4600
5	24	19.2	41	306	3005	4120
6	30	31.3	61.5	510	3010	3890
7	30	24	60	510	3370	4080
8	34	21.7	42	407	3125	4380
9	35	19.5	54.5	327	2695	3730
10	40	24.1	44.5	681	3080	4150
11	40	30.8	93.5	682	2860	3730
12	42	19.4	39	525	3030	3925
13	42	23.6	72	536	2805	3675
14	44	23.7	56.8	536	2750	4600
15	45	27.2	66	676	3180	4560
16	45	21.4	61	525	2870	4050
17	46	20.7	35.5	510	3115	3945
18	46	19.4	30.5	550	2990	3835
19	48	24.5	53.5	495	3060	4460
20	48	26.2	55	798	2780	3715
21	50	22.9	53.6	502	3025	4315
22	52	21.2	20	455	2900	4120
23	57	21.4	39	448	2990	3975

Case No.	PMI (hr)	Mg (mg/l)	Ca (mg/l)	K (mg/l)	Na (mg/l)	Cl (mg/l)
24	60	27.8	63.7	727	2960	3820
25	64	23.8	56	677	2800	3820
26	66	24.9	89.5	740	3100	4120
27	68	21.2	34.5	565	3170	4010
28	68	23.7	43	623	2880	3765
29	70	20.7	62	547	2785	4210
30	72	24.2	45.6	740	2990	4210
31	72	20.5	48.5	1700	3020	3890
32	72	22	14.4	562	3020	4150
33	72	23.8	52.8	626	3000	4120
34	72	20.8	62	496	2800	3855
35	76	23.4	59.5	587	2810	3765
36	78	30	74.5	895	3080	4200
37	78	24.4	45.4	691	2900	4210
38	80	33.8	71	968	2820	3890
39	92	36.5	47	746	2715	3890
40	92	29.6	51	762	2950	4380
41	94	24.2	74.5	1167	3230	3890
42	96	24.5	74	740	2910	3710
43	98	26.9	88.5	855	2625	3840
44	100	30.3	64	846	2560	3360
45	100	25	56	694	2805	3465
46	100	29.4	72	967	2710	4315
47	110	27.3	56	907	2725	3695
48	115	25	46	750	3020	3360

TABLE 2

ELECTROLYTE CONCENTRATIONS IN THE VITREOUS HUMOUR OF FRESH WATER AND SALT WATER DROWNING VICTIMS

Case	Imm.T	PMI	Mg	Ca	K	Na	Cl
1	0.20	24	24	16	410	2780	3800
2	0.30	44	21.3	53	640	3230	4035
3	0.75	120	23.9	62.5	733	1910	
4	1	20	22.4	30	304	2820	3960
5	1	40	25.6	50.5	360	3185	4385
6	1.5	67	20.7	61	573	2815	
7	1.5	2	18	44	640	2920	3710
8	2	18	18.4	51.5	332	3035	4225
9	2	40	23.8	61.5	326	2800	3570
10	3	22	23.9	61	397	3050	4015
11	3	30	20.4	70	385	2940	4460
12	3	42	17.7	58.5	300	2750	
13	4	48	22.4	51	567	2920	4775
14	4	70	21	50	630	2730	4030
15	5	24	21.4	59	474	2740	3070
16	5	57	13.6	43	271	3060	4440
17	5	27	22.7	65.5	510	3250	3840
18	6	30	21.9	36	299	2840	4135
19	7	48	20.8	16.5	284	3060	4155
20	7	66	23.4	61.5	415	2980	4210
21	8	66	21.4	66.5	472	2660	
23	8	96	19.2	48	685	3405	4080

Case	Imm.T	PMI	Mg	Ca	K	Na	Cl
24	9	115	28.9	28.9	740	2875	4315
25	12	20	21.3	40.7	332	3070	4440
26	12	80	20.7	57.5	455	2510	
27	14	110	16.4	50	965	1960	3340
28	15	42	23.7	50	498	2710	3610
29 sw	17	27.5	40.8	76.5	533	3590	
30	18	78	20	33	760	2530	3890
31	20	60	18.6	51.5	480	2260	
32	24	50	50	15.2	60.5	389	2660
33	29	100	18	51	919	2660	3715
34	65	110	22.2	39	697	2550	3110
35	65	110	21.6	35.5	695	2080	3270
36	70	115	21.4	52	723	2535	2950
37	72	120	25.6	78.5	792	2380	2755
38	72	120	17.5	12	595	2660	3620
39	90	120	19.8	52.5	596	2020	
40	96	136	18.3	44.5	662	2480	3715
41	96	144	23.5	56	810	1875	2880
42	130	150	32	75	1062	2620	4070
43 sw	140	164	35	170.5	1038	5970	10120

TAB. 3. Range and mean of the values of Imm. T., PMI, Mg, Ca, K, Na, and Cl obtained from Control, fresh water and salt water drowning cases.

	CONTROL	FWD	SWD
No. of Cases	48	41	2
Imm. T.			
Range		0.2-130	17-140
Mean		24.8±34.3	78.5±87.0
PMI			
Range	8-115	2-150	27.7-164
Mean	59.8±27.9	70.4±41.0	95.8±96.4
Mg			
Range	19.2-36.5	13.6-32	35-40.8
Mean	24.5±3.9	21.3±3.4	37.9±4.1
Ca			
Range	14.4-93.5	12.0-78.5	76.5-170.5
Mean	56.3±56.3	49.6±15.2	123.5±66.5
K			
Range	306-1167	271-1062	533-1038
Mean	631±181.6	544.4±202.6	785.5±357.1
Na			
Range	2560-3370	1875-3405	3590-5970
Mean	2933.2±167.2	2707.9±373.7	4780±1682.9
Cl			
Range	3360-4600	2755-4775	
mean	4014.4±301.4	3833.3±508.6	

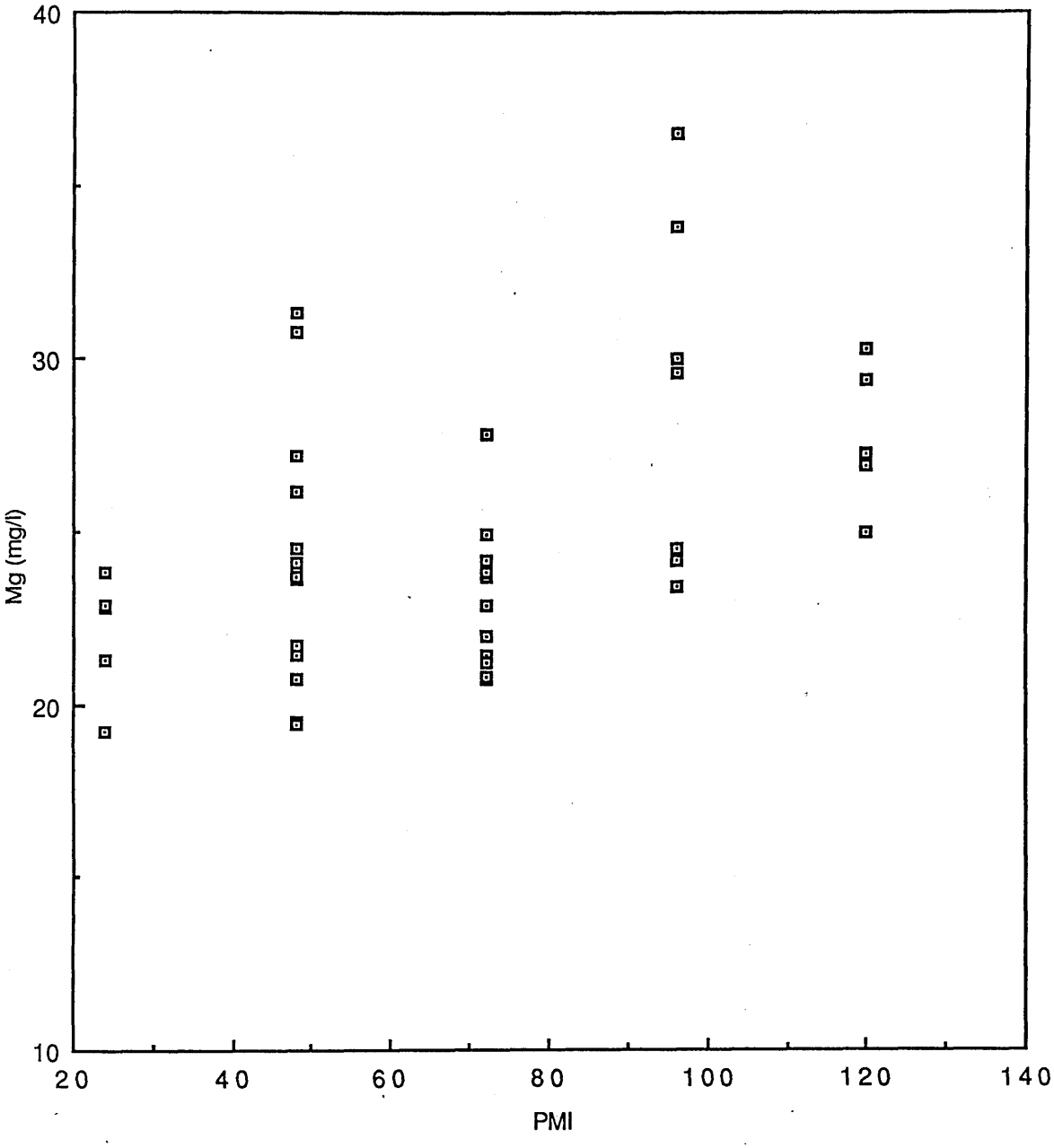

Fig. 1 A. Concentration (mg/l) of Mg in postmortem vitreous humour of control cases plotted in successive 24-h postmortem interval periods

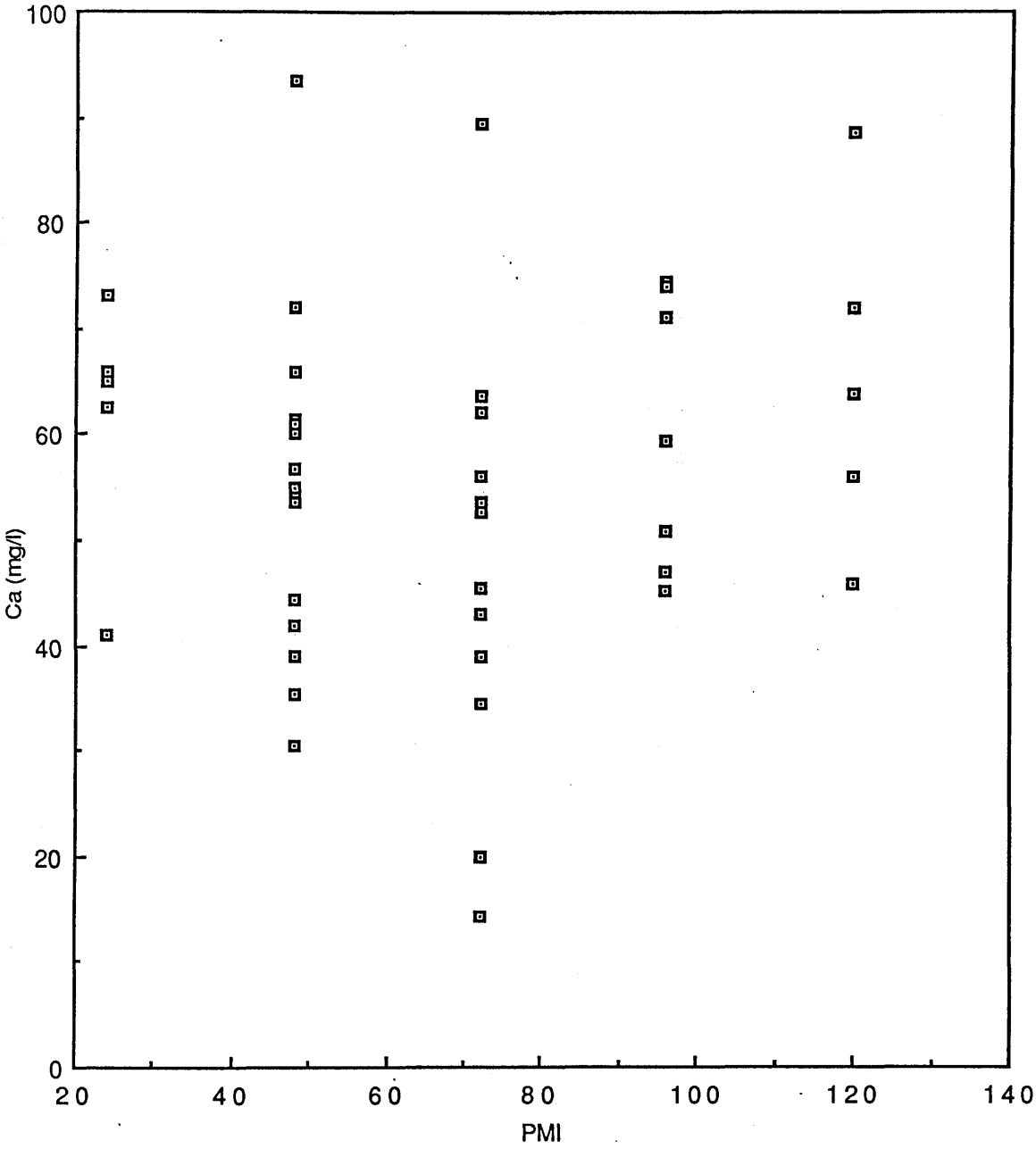

Fig. 1 B. Concentration (mg/l) of Ca in postmortem vitreous humour of control cases plotted in successive 24-h postmortem interval periods

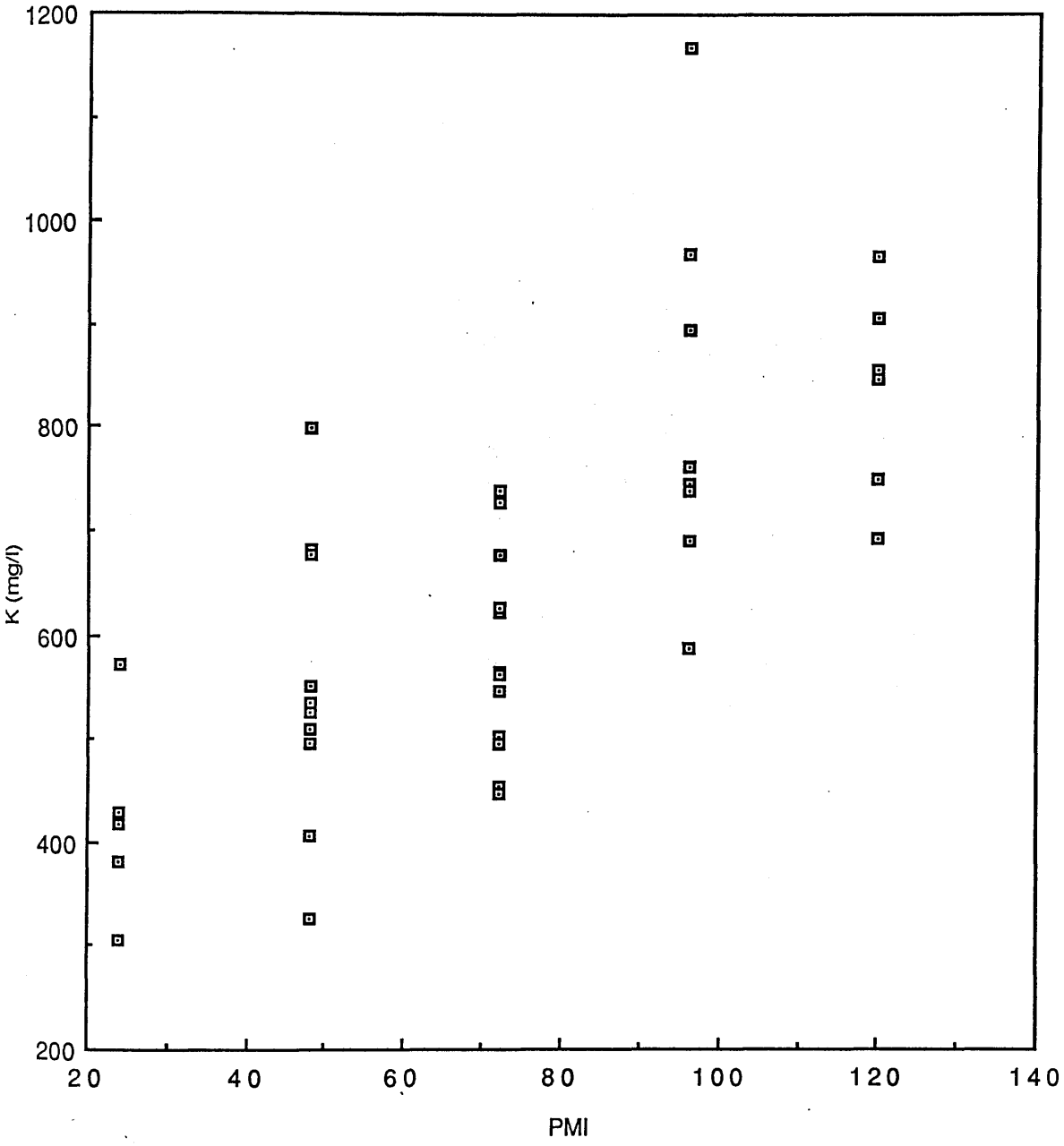

Fig. 1 C. Concentration (mg/l) of K in postmortem vitreous humour of control cases plotted in successive 24-h postmortem interval periods

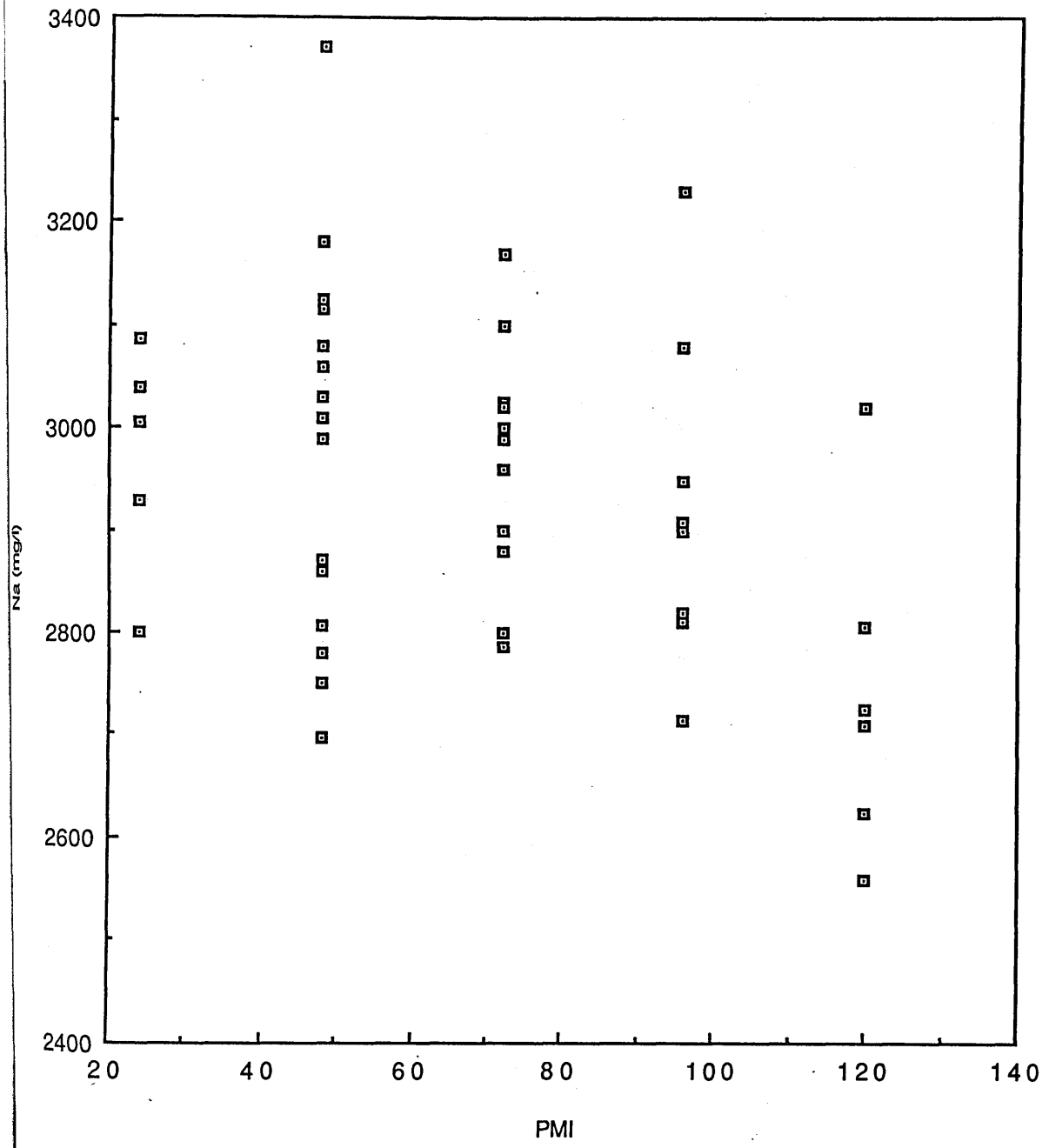

Fig. 1 D. Concentration (mg/l) of Na in postmortem vitreous humour of control cases plotted in successive 24-h postmortem interval periods

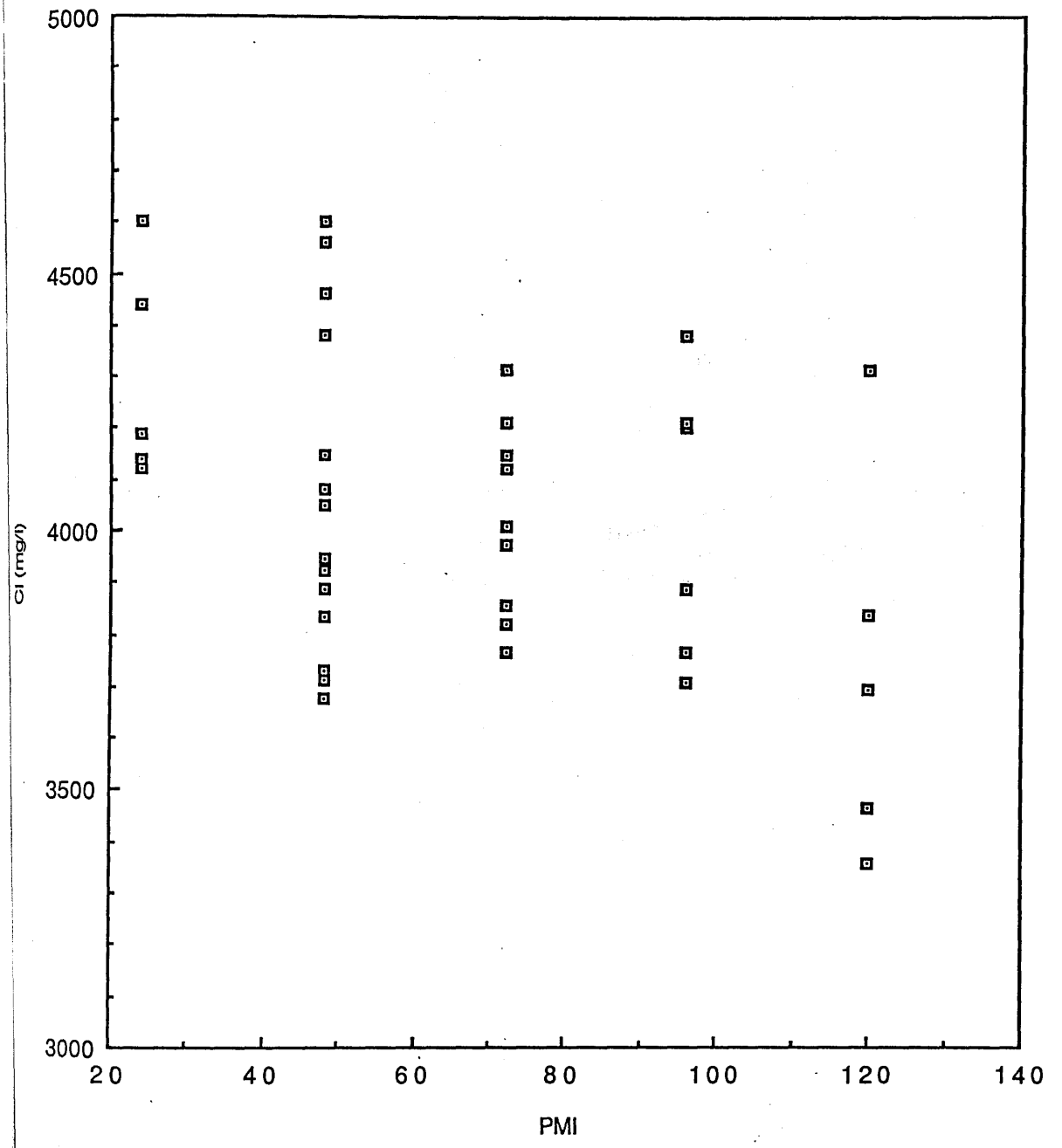

Fig. 1 E. Concentration (mg/l) of Cl in postmortem vitreous humour of control cases plotted in successive 24-h postmortem interval periods

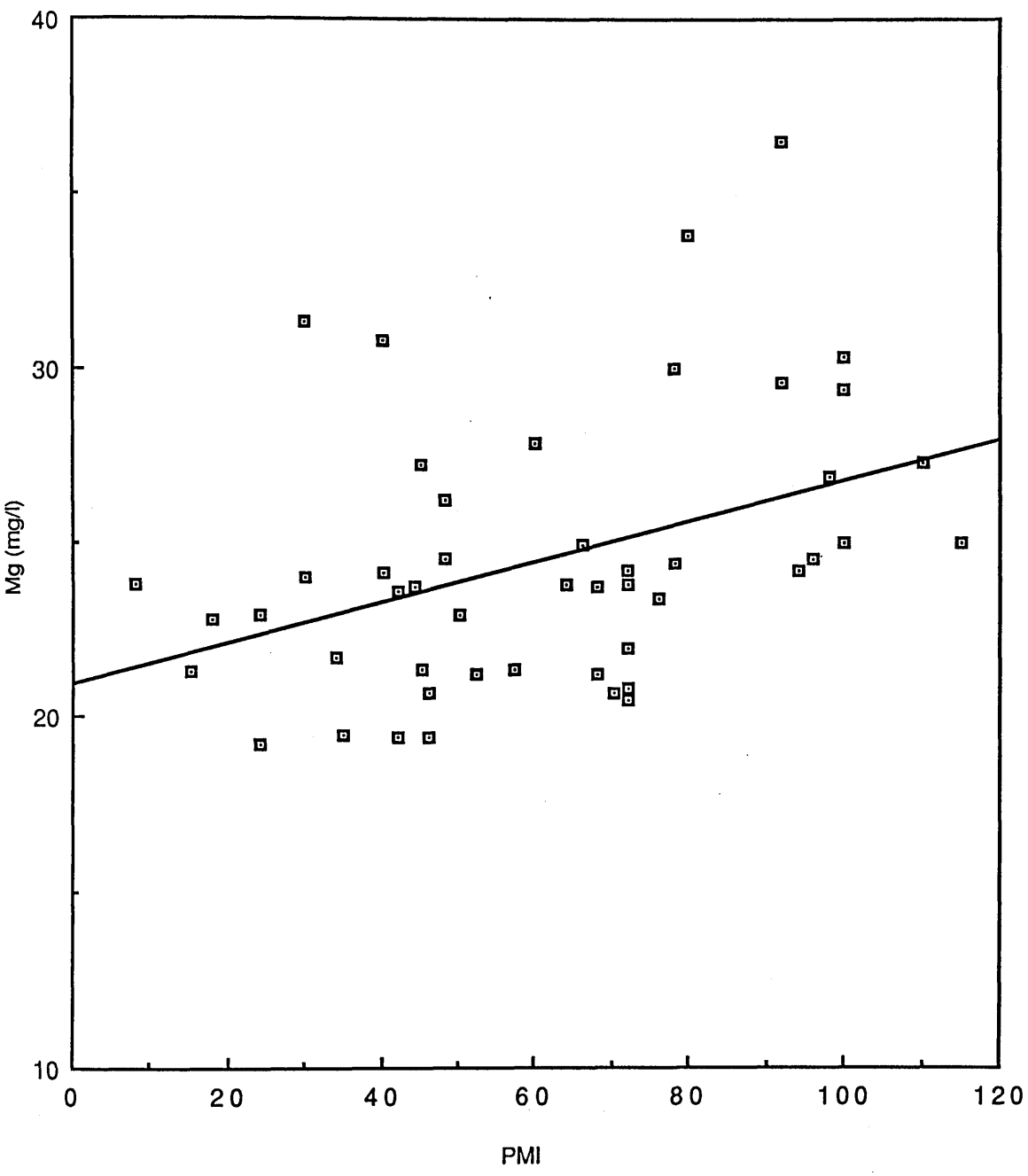

Fig. 2. Values for concentration of Mg (mg/l) in vitreous humour of controls in relation to postmortem interval (h).

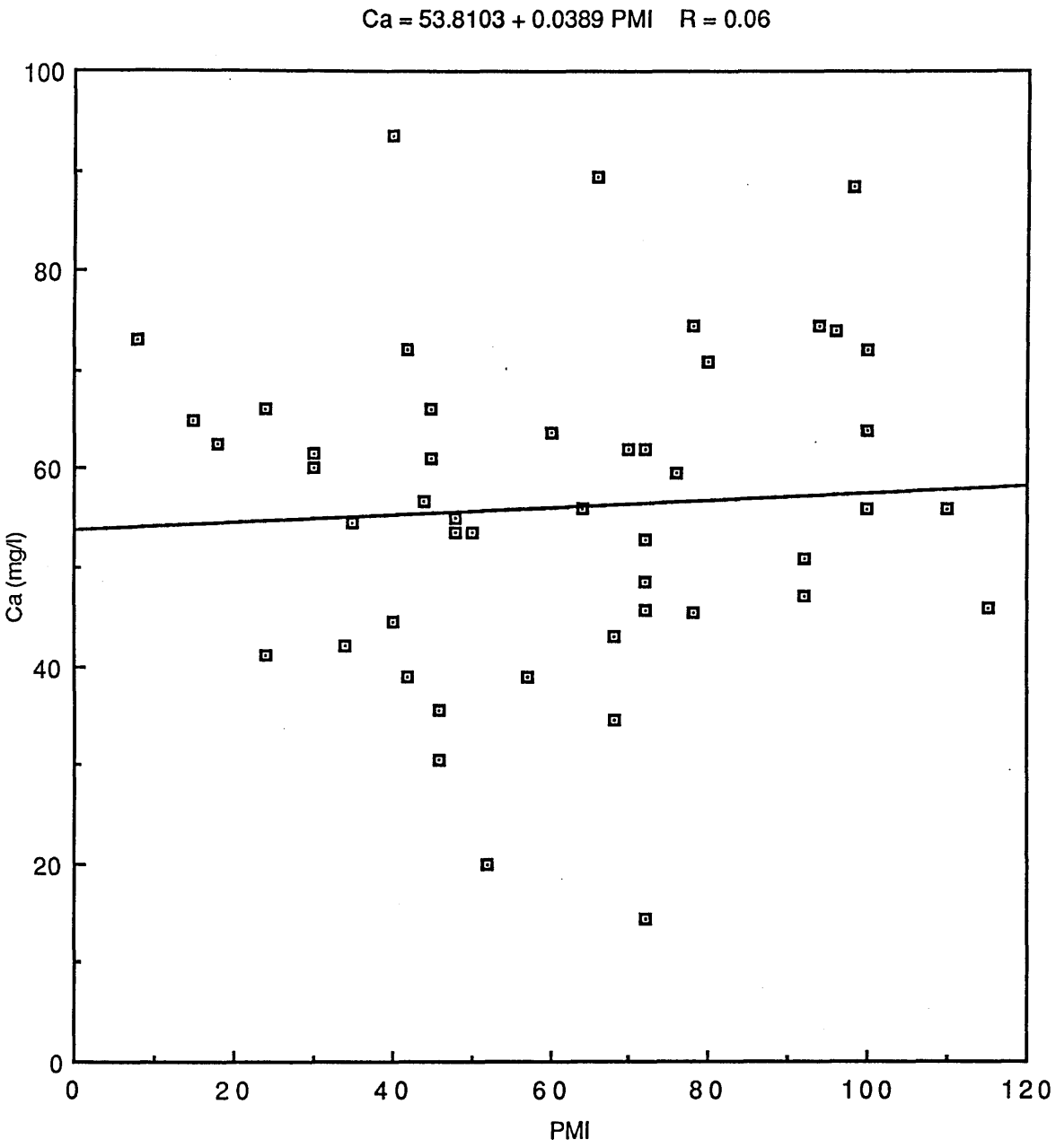

Fig. 3. Values for concentration of Ca (mg/l) in vitreous humour of controls in relation to postmortem interval (h).

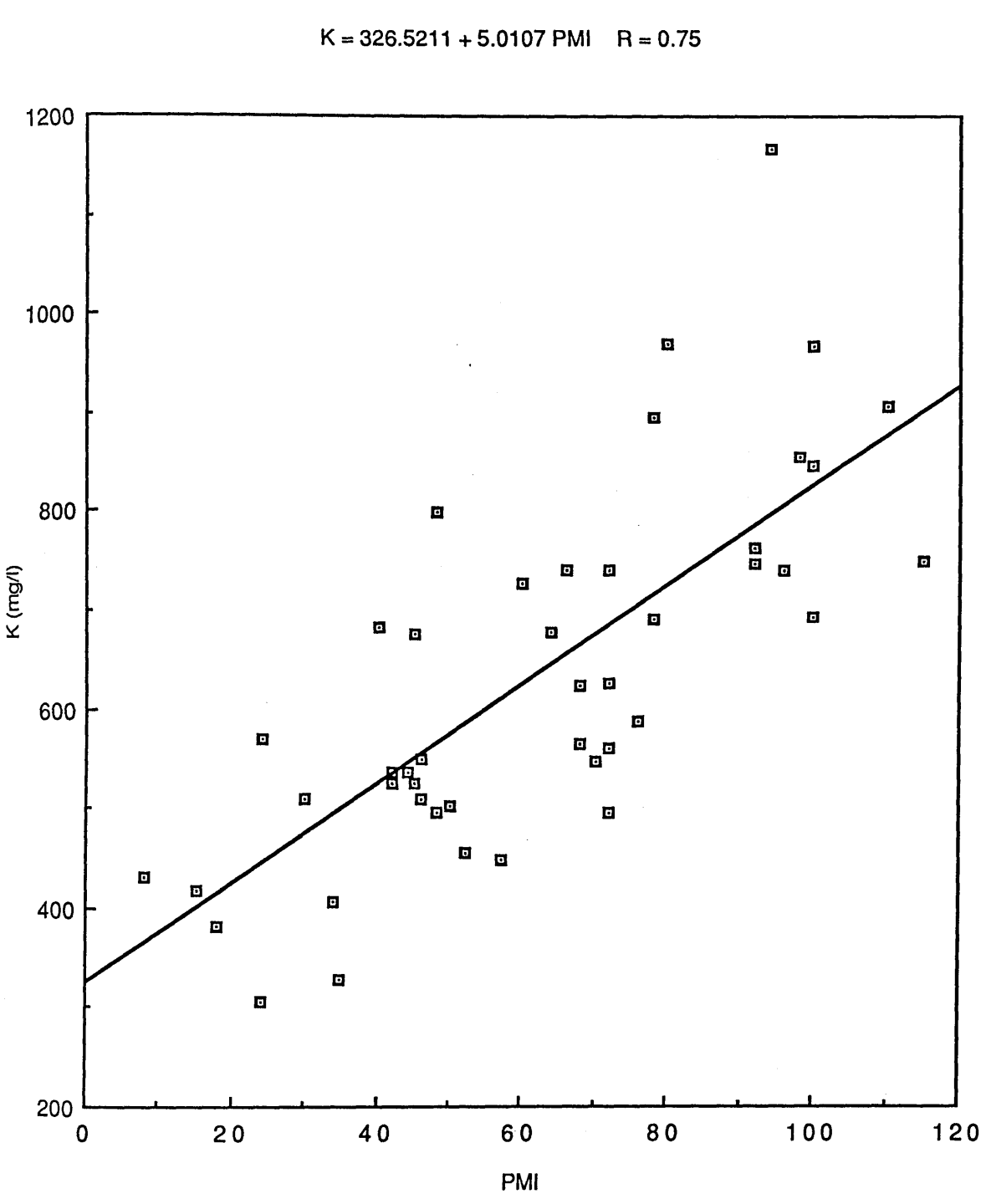

Fig. 4. Values for concentration of K (mg/l) in vitreous humour of controls in relation to postmortem interval (h).

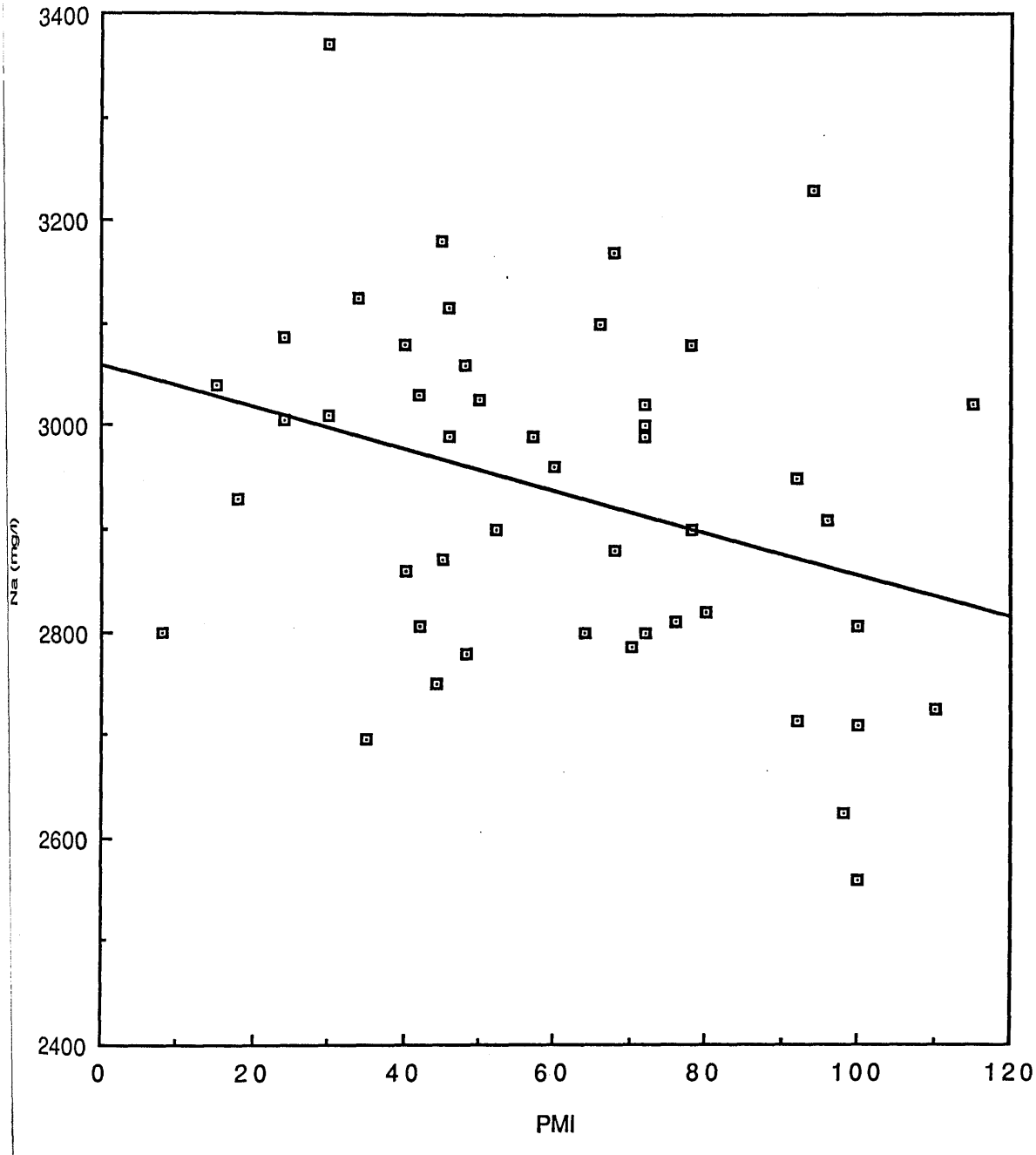

Fig. 5. Values for concentration of Na (mg/l) in vitreous humour of controls in relation to postmortem interval (h).

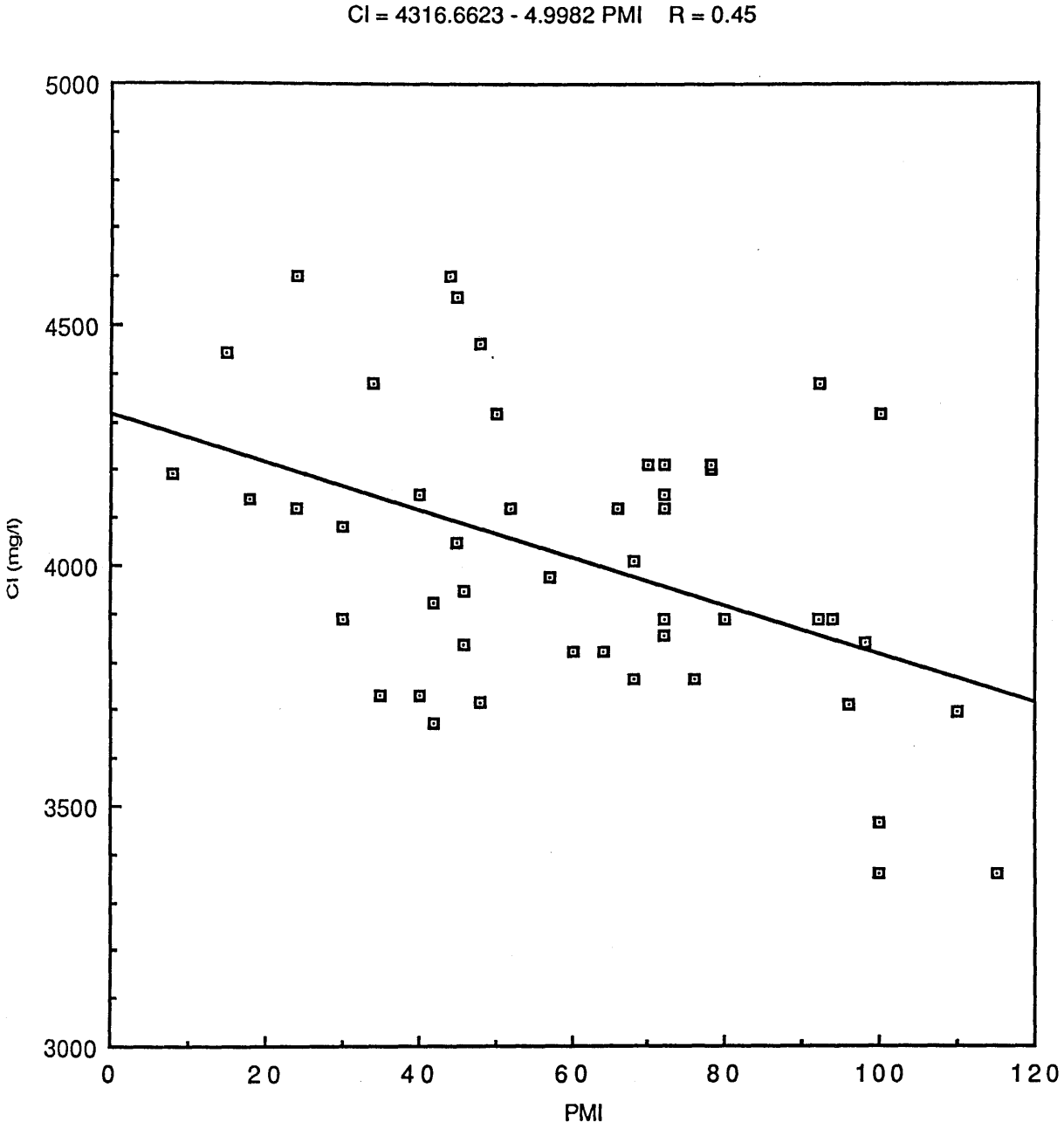

Fig. 6. Values for concentration of Cl (mg/l) in vitreous humour of controls in relation to postmortem interval (h).

which (R) represents the coefficient of correlation and (S) represents standard error of estimate of the regression line.

PMI=- 7.43+2.97 Mg	R= 0.40	S= 24.8	(1)
PMI= 54.47 + 0.11 Ca	R= 0.07	S= 27.3	(2)
PMI= -9.24 + 0.11 K	R= 0.75	S= 18.2	(3)
PMI= 218.46 - 0.05 Na	R= -0.33	S= 25.8	(4)
PMI= 221.38 -0.04 Cl	R= -0.45	S= 24.5	(5)

From these correlation equations, the best correlation was obtained between postmortem interval and K with Coefficient correlation R= 0.75, and standard error of estimated postmortem interval around the regression line S= 18.2, which is slightly better than other electrolytes, Mg(24.8), Ca(27.3), Na(25.8) and Cl(24.5) with correlation coefficients of 0.40, 0.07, 0.33 and 0.45 respectively.

In using mean electrolyte values from 24 hours period interval, the correlation-coefficient of the electrolytes with postmortem interval are more significant than when using individual electrolyte values. The regression equations with coefficients correlation and standard error of estimated of the mean are as follow ;

PMI= 191.52 + 10.24 Mg	R= 0.84	S= 21.5	(6)
PMI= -18.25 + 1.39 Ca	R= 0.25	S= 38.4	(7)
PMI= -56.55 + 0.18 K	R= 0.97	S= 9.9	(8)
PMI= 852.65 - 0.27 Na	R= -0.79	S= 24.3	(9)
PMI= 627.10 - 0.14 Cl	R= -0.92	S= 15.7	(10)

Furthermore the prediction of postmortem interval from

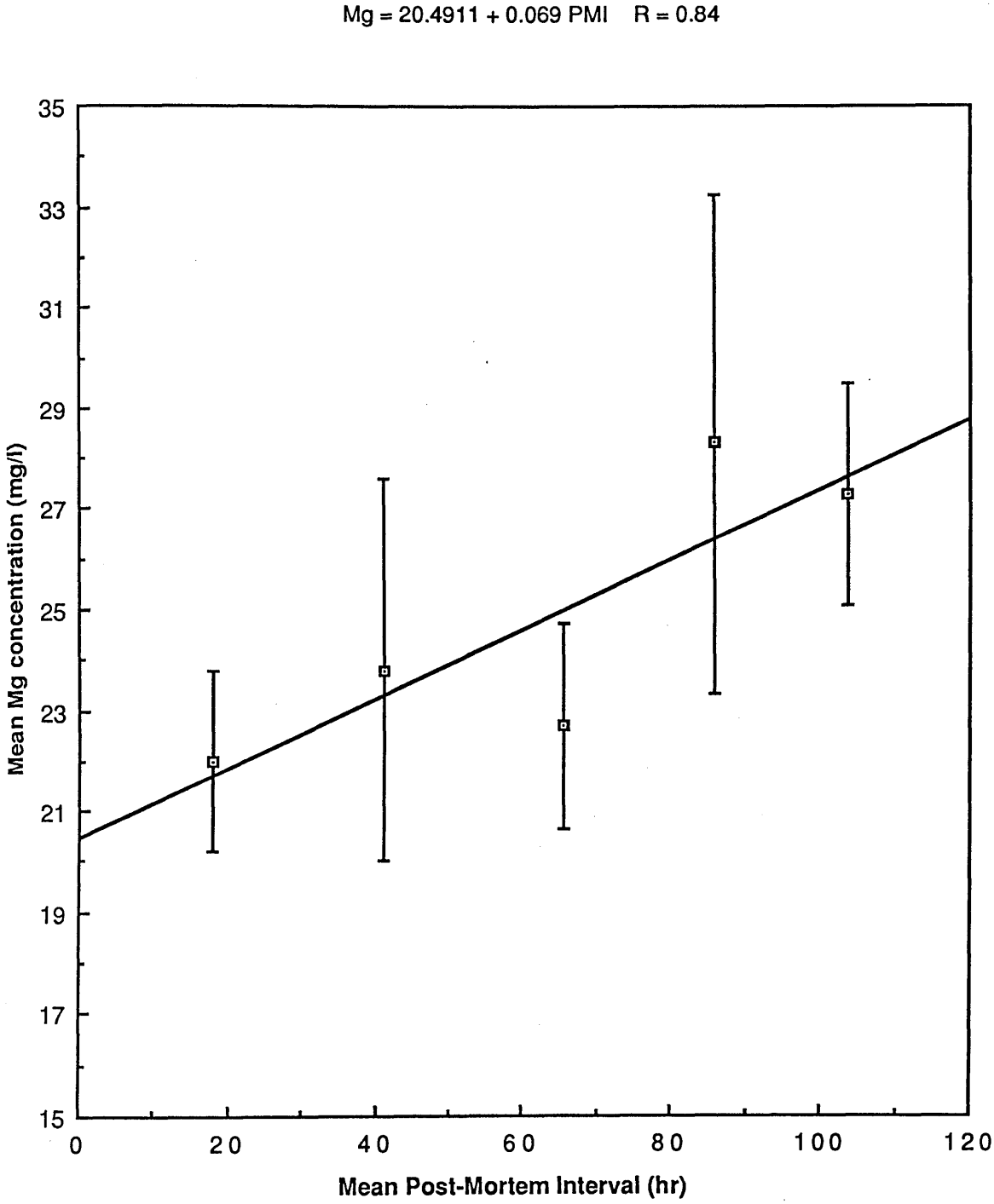

Fig. 7. Mean concentration (±1 S.D.) (mg/l) of Mg in postmortem vitreous humour of controls for the mean PMIs of the successive 24-h PMI period.
Mg = 20.49 + 0.069 PMI (R = 0.84).

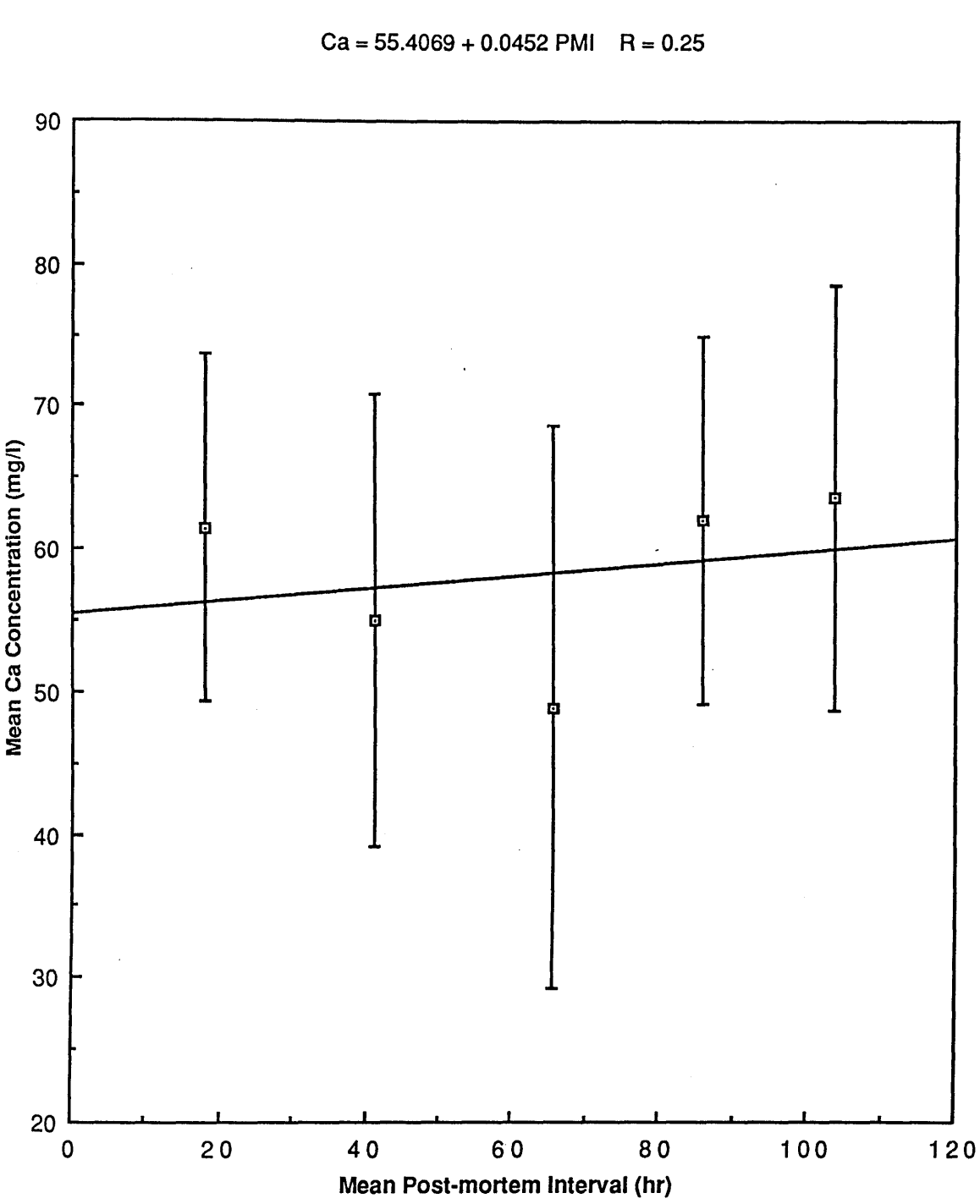

Fig. 8. Mean concentration (±1 S.D.) (mg/l) of Ca in postmortem vitreous humour of controls for the mean PMIs of the successive 24-h PMI period.
Ca = 55.4 + 0.045 PMI (R = 0.25)

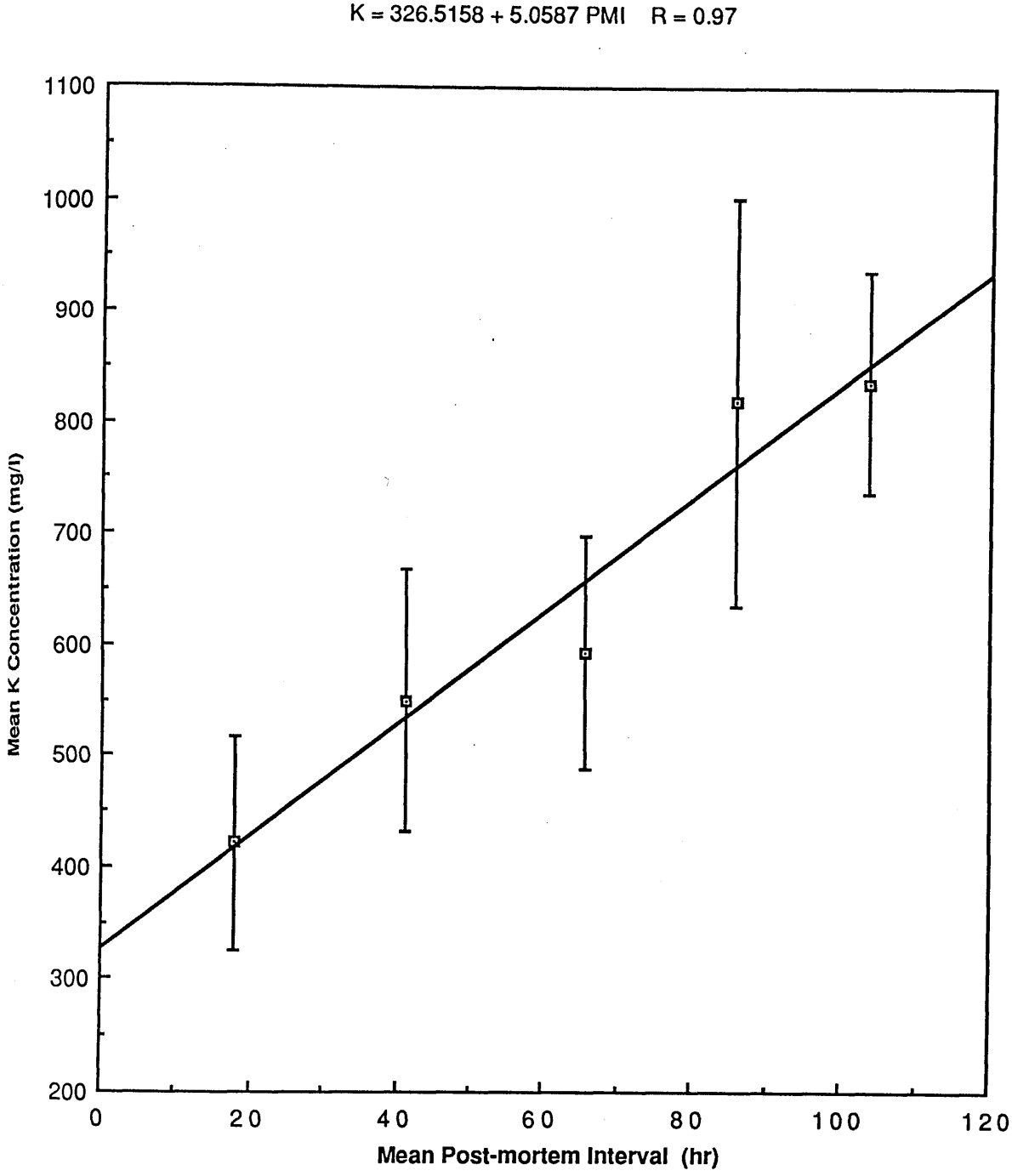

Fig. 9. Mean concentration (±1 S.D.) (mg/l) of K in postmortem vitreous humour of controls for the mean PMIs of the successive 24-h PMI period.
K = 326.5 + 5.0 PMI (R = 0.97)

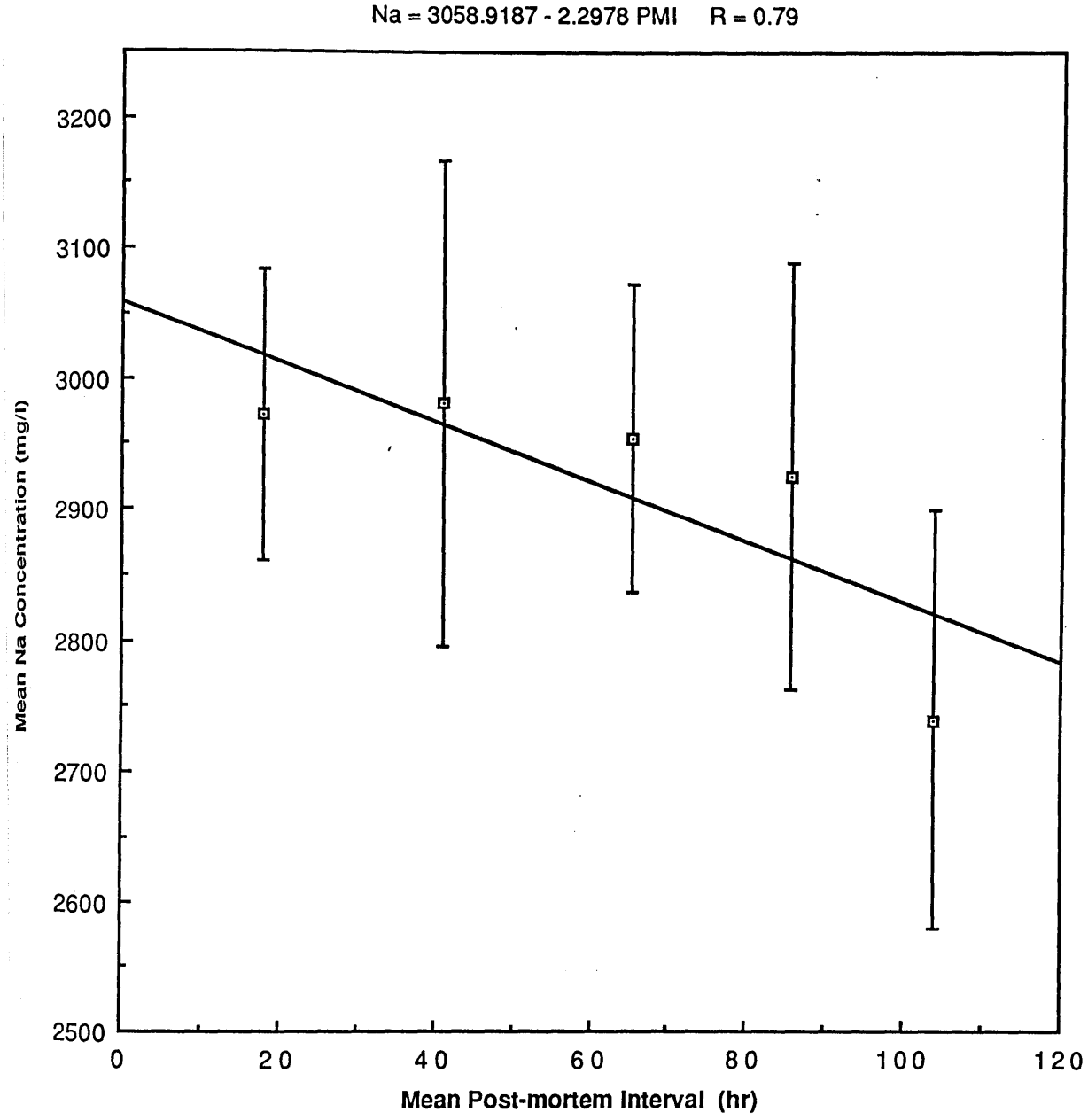

Fig. 10. Mean concentration (±1 S.D.) (mg/l) of Na in postmortem vitreous humour of controls for the mean PMIs of the successive 24-h PMI period.
Na = 3058 - 2.297 PMI (R = - 0.79)

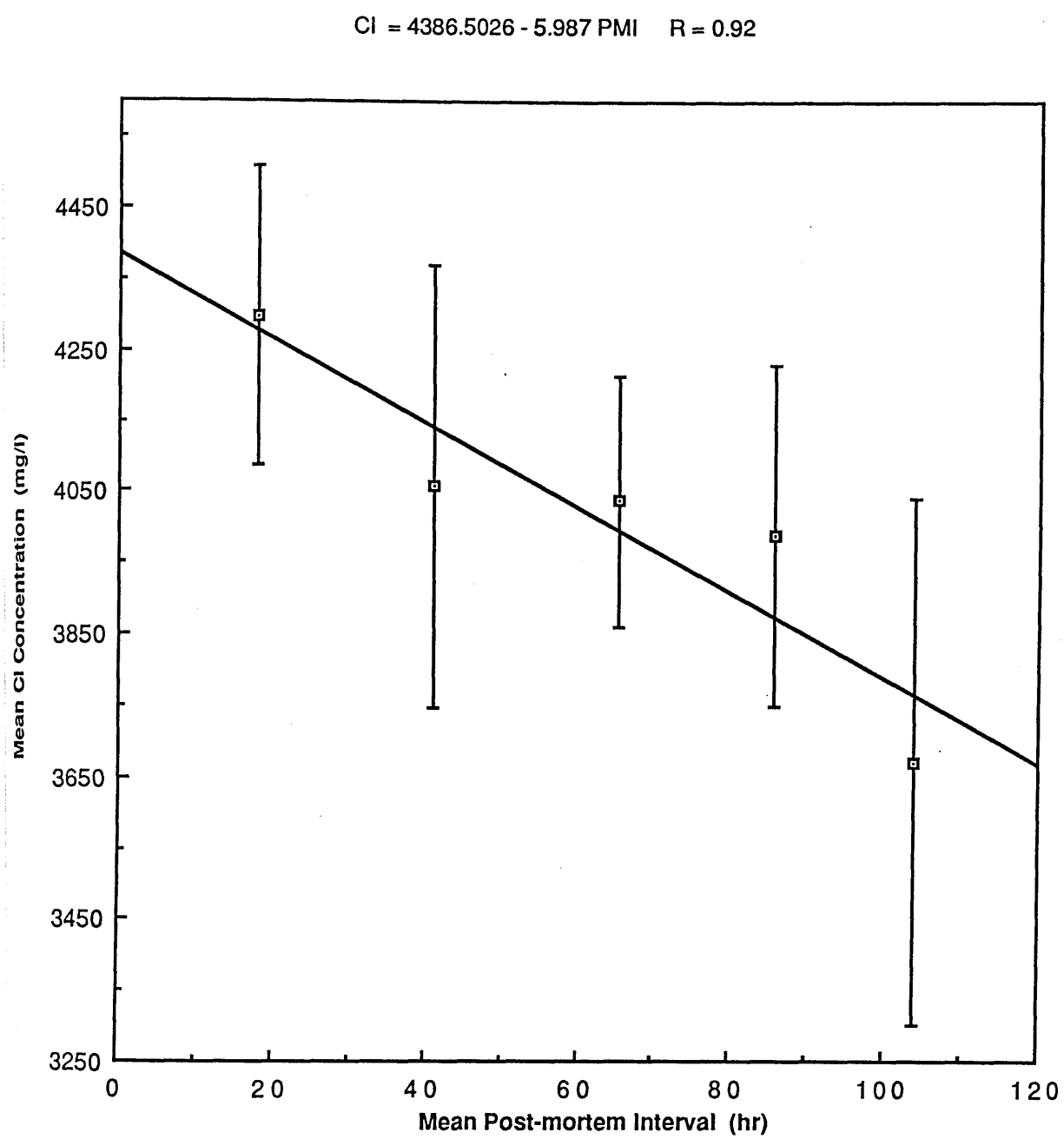

Fig. 11. Mean concentration (±1 S.D.) (mg/l) of Cl in postmortem vitreous humour of controls for the mean PMIs of the successive 24-h PMI period.
Cl = 4386 - 5.98 PMI (R = 0.92)

individual values are enhanced considerably by using multiple regression equations. The following equations show significant correlation with enhanced prediction of postmortem interval;

PMI= 1.7 - 0.7 Mg + 0.1 K R^2= 56.4 S= 18.3 (11)

PMI= 93.7 + 0.1 K - 0.01 Cl R^2= 59.6 S= 17 (12)

PMI= 99.2 + 0.1 K - 0.01 Na R^2= 59.4 S= 17.4 (13)

By applying the multiple regression equation on mean 24-hour vitreous electrolyte values enabled prediction of postmortem interval within a shorter time, as in case of using Mg v K, K v Cl and K v Na with postmortem interval, as is shown in the following equations ;

PMI= 89.0-9.6 Mg + 0.3 K R= 0.95 S= 1.9 (14)

PMI= 178.6+ 0.1 K- 0.1 Cl R= 0.89 S= 9.1 (15)

PMI= 129.1 + 0.2 K - 0.1 Na R=0.86 S= 10.8 (16)

Other less significant but noteworthy correlation are those between :

Mg and K (i:R = 0.64 ; m:R = 0.95)

Mg and Ca (i:R = 0.43; m:R = 0.59)

K and Ca (i:R = 0.40 ; m:R = 0.40)

Na and Cl (i:R = 0.40 ; m:R = 0.89)

3.2. DROWNING DATA :

Forty three bodies recovered from water were submitted for analysis of vitreous humour electrolytes. The postmortem interval and immersion time in these cases were measured, relying on many sources to obtain the required information. These sources included witnesses, police and circumstantial evidence. In the 41 cases of

drowning the immersion time ranged between 0.2 h. to 150 h with a mean value of 24.3 (±34.3 h), while for postmortem interval, it was 2-150 h (70.4±41.0). In the 2 cases of salt water drowning the immersion time was 7 h and 140 and postmortem interval 27.7 h and 164 h respectively (Tab.2).

Mg, Ca, K, Na, and Cl in vitreous humour were examined in all cases of drowning except that 8 fresh water drownings and the 2 salt water drowning which were not examined for Cl, due to the shortage of samples. The details of drowning cases included immersion time, postmortem interval and the individual values of the electrolytes are shown in table-2.

4. DISCUSSION :

4.1.MAGNESIUM :

Vitreous humour would appear to be the most practical source of postmortem material for magnesium determination. The body Mg is primarily intracellular and in bone. The measurement of serum Mg has been considered as being the most practical. However, serum Mg concentration increases at an unpredictable rate after death due to increase of the membrane permeability and cell destruction. It cannot be correlated accurately with antemortem values. In cerebrospinal fluid, Mg values show unpredictable postmortem variability (29).

The concentration of Mg in vitreous humour varies according to the age of the person e.g. a range of 18-30

mg/l. was reported in a series of 24 adults compared with a range of 24-48.8 mg/l. obtained from a series of 20 infants (159). Therefore, the level of magnesium are found to be both higher and age related in young children(12,13,159).

Data on vitreous humour magnesium is sparse for adults, Sturner (159) reporting a range of 18-30 mg/l. (n=24), Sturner et. al. (158) a maximum of 39.5 mg/l. (postmortem interval ≤ 36 h; n= 15). Others reported an average value of 26.0 mg/l. (postmortem interval 7-15 h ; n= 53). In other study Wheeler et. al.(183) found a range value of 7-29 mg/l. (mean=15.8 mg/l.) for 27 alcoholics (postmortem interval 6- 32 h) and a not significantly different 5-25 mg/l. (mean 17.2 mg/l.) from 27 non- alcoholic (postmortem interval 3.5-31 h). These data are broadly comparable with the 19.2-36.5 mg/l. (mean 24.5 ± 3.9 mg/l.) range found in this study.

As vitreous humour magnesium levels are known to be higher and age related in young children, Workers (13) found significant differences in vitreous humour magnesium between premature, full time infants and neonates (age 1 h. to 8 days ; mean 58 mg/l. ± 7 mg/l) relative to older children (2 months to 13 years; mean 33 mg/l.± 5 mg/l.). Sturner (159) obtained a range of 24-47 mg/l. for 20 infants while Swift et.al.(164) recorded a maximum of 70 mg/l., although the level declined to 20-30 mg/l.by 4 years of age.

The use of vitreous humour magnesium in detecting

antemortem metabolic abnormalities is unproven. A possible relationship between sudden unexpected infant death and deficiencies in body magnesium levels(as reflected in vitreous humour magnesium) was suggested by Sturner (159) but was not subsequently confirmed by Blumenfeld et.al.(11) who found a mean magnesium level of 43.3 mg/l.± 6.5 mg/l. for 27 infants (SIDS) but only 32.3 mg/l.± 6.3 mg/l. for normal controls. Although this difference was statistically significant, it was not considered to be of clinical importance in terms of positively indicating, or determining the cause of SIDS.

Magnesium, although less extensively studied, has been considered with respect to unexpected infant death (11,12,159) or as a possible aid in the diagnosis of drowning or in estimating the immersion time of drowning victims.(4).

Figure (2) shows the relationship between magnesium and postmortem interval, in which a significant correlation is drawn from the regression equation of the two variables (R= 0.40). Though there is no consistent relationship between the two variables, but from the gradient (0.058 mg/l per h), the concentration of magnesium showed some increase with increasing postmortem interval. By using the mean values of 24-h periods there is not much enhancement in the trend of Mg increase (0.069) though the correlation coefficient between Mg and postmortem interval is significant (0.84).

4.2. SODIUM :

It was concluded that changes in concentration of Sodium, as well as other constituents of intraocular fluid are too variable to be used in investigation of the postmortem interval (83,101). As shown in Fig.-5, the wide scatter pattern of the values of Na in relation to postmortem interval cannot be used as an indicator to measure the time elapsed after death. However the mean values of 24 h periods of Na showed relatively consistent decline with increasing postmortem interval (Fig.10). Sodium was, also, found of little value in determining sudden infant death syndrome (SIDS) or in estimating the time of death in these cases. However, it might be a useful addition in the postmortem examination of children, because the results could indicate antemortem serum abnormality which would not be disclosed by conventional postmortem examination of tissues (11).

It was demonstrated that sodium remains stable postmortem (11) and neither time nor temperature appears to influence changes of its concentration in the vitreous humour. The stability in sodium is thought to last 24 hours postmortem after which a somewhat inconclusive decrease is noted (85).

The postmortem stability of vitreous sodium concentration was noted by Naumann (120) and re-affirmed by Leahy et.al.(100) who found vitreous sodium concentration to be stable up to 30 hours after death. Very similar results were obtained in this study.

4.3. POTASSIUM :

The most significant correlation is that of K with postmortem interval. The regression coefficient for this correlation is 0.75 for 48 individual values of potassium.

Naumann (120) was the first to show that the vitreous potassium content rose postmortem and he suggested that lysis of cell membrane in the vascular choriod was the source of potassium. This was later substantiated by other workers (80,83). Attention was also drawn to the vascular choroid and retinal lining cells as potential sources of the ions (120).

Despite the reported differences in the utility of this method i.e. estimation of potassium in vitreous humour, there seems to be an agreement on several points ;

1. The vitreous potassium level rises postmortem more rapidly in the first 6-12 hours but in a fairly linear fashion after 24 hours, reaching a maximum at 100-120 hours (27,74).

2. The samples are stable for 24-36 hours refrigerated at 4C° and for longer period frozen at -20C°.

3. The level of potassium is not influenced by the presence of cataract, previous cataract, surgery or previous embalming (unless the embalming fluid contains significant quantity of potassium) (160).

4. Results are liable to be distorted, however, by some intrinsic eye diseases, previous removal of the brain at autopsy more than 24 hours,earlier, or collection of

inadequate sample of a volume less than 0.5 ml.(159,160).

However the value of K permits prediction of postmortem interval to within ± 18 h (1 S.D.) (Fig. 4). This time interval is too broad to be of use in ascertaining postmortem interval, even in cases of acute trauma with postmortem interval within 6 hours. However, greater success in predicting the postmortem interval was obtained by using the mean values. Sturner and Gantner (160) were able to calculate the post-mortem interval with an acceptable limit of confidence, finding that the standard error did not grow with increasing time post-mortem. They found the most useful interval for this technique was the period 24-100 hours post-mortem. Lie (101) was able to confirm these results with great precision, and Adjutantis and Coutselinis (3) were able to use the technique by applying a single potassium measurement within 12 hours of death. Others, however, found that this technique was not usable, or that the confidence interval of the time of death lay in the range of ± 10-26 hours (2 S.D.) and increased with time post-mortem (2,11,28,74,80,100).

Consideration of possible sources of error may lead to ways to eliminate or minimize some of them. Some of reported discrepancies may be due to different analytical techniques. Some, no doubt, result from biological variation in the chemical constitution of the vitreous, and there is no way to eliminate or estimate this error in the baseline. Hospitalized patients showed a definite correlation between the antemortem serum and post-mortem

vitreous potassium content, and it is generally accepted that the ionic constitution of the vitreous depends on the plasma (37,160).

From the results obtained, these pertinent facts emerge:- 1. The relation of increase in K to the time postmortem is arithmetic.

2. The standard error does not increase as the death interval lengthens.

The explanation for these two observations may be that the cell system responsible for increase of K is free from (or soon loses) enzymatic dependency after death, and reverts to a somewhat less than a true biological system.

One may postulate that brief enzymatic activity shortly after death contributes to the "broad baseline" in any group of patients. Thus, a small but perhaps relevant error is introduced, which is not compound as time progresses.

It may be attributed to intraocular autolytic processes. As the estimated value of the potassium content of the vitreous body was found to be 180-190 mg/l. It was also noted that the vitreous layer contained far higher quantities of K than the estimated values.

It is thought that the increase in the K value is limited and tends to show a "saturating limit" conditioned by the potassium supplies which can diffuse into the vitreous body from the surrounding tissue. The time required to achieve such a limit is about 12 hours

(33). However in the current study no apparent limit was achieved over a period of 115 hours of postmortem interval.

4.4. CHLORIDE :

The declining pattern of Cl values with increasing postmortem interval (Fig.6) contradict the idea of its stability for any considerable time after death. As shown in Figs.(6) and (11) there is a significant decrease of Cl with gradients of 4.97 mg/l per h for the individual values and 5.98 mg/l per h for mean values. However, like other constituents of the vitreous humour, it cannot be used with accuracy for the estimation of postmortem interval. The measurable fluctuation in chloride concentration does not appear to reflect accurately the agonal values of chloride. This might be due to the phenomenon of individual biological variation which restricts using these electrolytes for estimating the postmortem interval or even in diagnosing the cause of death in certain conditions. In investigation of SIDS cases, chloride as well as Mg were found to be higher in these cases. However chloride is of little values, as are other constituents of vitreous humour, in differentiating children dying from SIDS and those dying from other causes, or to give any information or pertaining to the aetiology of SIDS (11). Also, Chloride concentration in vitreous humour failed to show any significant correlation with postmortem interval in these cases.

The chloride ion is one of the components that shows the "decomposition pattern" postmortem, which results from a prolonged postmortem interval when the potassium concentration is markedly elevated while both sodium and chloride are both decreased. This pattern is caused by diffusion of ions between intracellular and extracellular spaces (2,3,29,74).

But one of the advantages of using chloride is that the concentration in vitreous humour proved not to be influenced by environmental temperature at the time of death (14).

4.5. CALCIUM :

The levels of calcium in vitreous humour range between 14.4-93.5 mg/l. with a mean of 56.3 ± 16.5 mg/l. These values are similar to those obtained by Nauman (120) (56-104, 72), Coe(72) (60-84, 68), Emery et.al.(49) (63-83), Defour(46) (57-84,69) and Farmer et.al.(51) (54.0-95.5, 66.5).

Defour (46) demonstrated a lack of correlation between serum and vitreous calcium concentration probably because of active transport mechanism within the eye rendering vitreous humour less useful for evaluating antemortem serum calcium concentration.

The literature on the usefulness of postmortem calcium determination is scant. Serum calcium, however , has been shown to rise rapidly after death, apparently because of loss of water from plasma to cells. Thus, postmortem measurement of serum calcium proved unhelpful for

predicting any significant change of calcium. On the other hand, postmortem changes in the vitreous calcium concentration occur slowly if at all.

Coe (27), and Blumenfeld et.al. (13) found no significant changes in vitreous calcium concentration with increasing postmortem intervals. Swift et.al. (164) recorded postmortem changes in 20 children by sampling the fluid from the two eyes at different postmortem intervals. In term of cases, the calcium concentration was higher in the second samples and in the other ten it was higher in the first samples.

In the current study there was no significant change in the calcium concentration with increasing postmortem times. The coefficient of correlation is 0.07 and slope of the regression line is nearly horizontal (0.041 mg/l.h.). These findings are similar to those of other workers (13,27,164), who found no significant postmortem changes in the vitreous calcium which could be related to the time elapsed after death.

The mean calcium concentration of 56.3 mg/l. is slightly lower than Coe's mean value (27) of 68 mg/l. and even lower than Naumann's mean (20) of 72 mg/l. It would seem that the methods used for measuring calcium concentration is not critical, as similar results for mean and postmortem stability have been reported using different techniques for the analysis of calcium in the vitreous humour.

4.6. ESTIMATION OF POSTMORTEM INTERVAL :

It is noted that there was sometimes a variation in the vitreous humour potassium concentration among control cases with the same post-mortem interval. There appeared to be a regular relationship between the concentration of potassium, sodium, and chloride in these cases. Regarding the correlation of postmortem interval with the vitreous humour electrolytes, there was a positive correlation between potassium and chloride concentrations (p <0.001). Also, there is a similar positive correlation between potassium and sodium. The control data show a tendency for the higher potassium concentration to be associated with lower sodium and chloride values

The large positive correlation between K and postmortem interval enables prediction of post-mortem intervals from individual measured and mean 24-hours period K values, using Equs.(3) and (8), respectively, and from corresponding values of two electrolytes via Eqns.(11), (12), (13),(14),(15), and (16). Overall the equations derived from individual and mean values by using simple regression correlation with postmortem interval, i.e.Eqns.(3) and (8) yield the more accurate prediction of postmortem interval, however those based on multiple correlation were superior.

In using mean 24-h period K values, only 22% of prediction of postmortem interval is within 3 h of true value, 45% within 6 h and 90% within 12 hours. The accuracy of prediction of postmortem interval seems to

deteriorate with increasing post-mortem interval.

The confidence limit obtained from Eqn.(3), in which the individual values of K have been used, was 18.2 h. While from Eqns. (11), (12) and (13), in which multiple regression equations of individual values of K & Mg, Cl & Cl, and K & Na are 18.3 h, 17 h, and 17.4 h respectively. Therefore, in using individual values, K is the best element in showing positive correlation with postmortem interval, in simple or multiple correlation with Mg, Na or Cl, there is only about 2/3 probability of the prediction of the postmortem interval falling within approximately 17-18 h of the true time of death. One of the factors for this uncertainty may be explained according to the individual biological variability (2,3,29,80), both in initial concentration (at PMI= 0) and in the rate of autolytic changes (at PMI >0). The other possible factor is the effects of environmental influences (e.g. on body temperature) between death and sampling (29), which was demonstrated in recent studies, when observed values of K in 7 of 13 fire-death cases were higher than those of predicted values. Vitreous humour potassium, despite the time-related release of intracellular potassium from retinal cells and vascular choroid, cannot be recommended as a perfect solution for estimating postmortem interval, though much enhancement in predicting postmortem interval has been achieved by using mean 24-h potassium values with simple or multiple regression equations.

Vitreous humour magnesium is even less helpful in

determining the post-mortem interval, the scatter of control Mg data precluding significant correlation with postmortem interval (Fig.1A and 7) and revealing an average increase of 0.058 mg/l in Mg concentration per h of postmortem interval. This is at the end of the published ranges of 0.03-0.09 mg/l per h and 0.1-0.22 mg/l per h.(4) based on magnesium measurement in the vitreous humour of each eye, collected from several individuals at two separate post-mortem intervals. The influence of external parameters as ambient temperature on post-mortem vitreous humour magnesium concentration was demonstrated by Farmer et. al.(51) who showed that despite the post-mortem interval shorter by an average 10 h, fire death data reveal an average magnesium concentration of 24.3± 3.3 mg/l compared with the 22.7 ± 3.1 mg/l of control cases.

The prediction of postmortem interval is enhanced by applying multiple regression equations of Mg & K (Eqn.11), Cl & K (Eqn.12), and Na & k (Equ.13) with individual values and equations (14), (15), and (16) of the mean values of the same electrolytes respectively. The confidence limits obtained from these equations are 1.9 h (Eqn.14), 9.1 h (Eqn.15) and 10.8 h (Eqn.16). These results confirm the significant correlations between K and Na or K and Cl as being related with postmortem interval.

4.7. DIAGNOSIS OF DROWNING :

Accurate estimation of time of death of a body found in water based on physical findings such as skin slippage or other putrefactive changes is made difficult by the fact that the rate at which these changes develop is affected profoundly by environmental temperature. Therefore biochemical methods of determining postmortem interval may prove useful in cases recovered from water when decomposition which has already started renders other methods and means of estimation of time since death totally inapplicable or futile.

Although vitreous humour magnesium is useless as an indicator of the post-mortem interval and, currently, of doubtful value in assisting the type of pathological inquiry of cause of death, it may well be of some use in the investigation of drownings. The measurement of various chemicals including electrolyte metals in other biological fluid has not been very successful in the diagnosis of drowning itself (143). It is suggested that the vitreous humour could be more useful than other biological fluids. This approach relies on changes produced in biological fluids metal concentration, either through processes subsequent to the direct intake of water or via diffusion of ions across boundaries between biological fluids and external water (4,16,24,143,160). In general, therefore, fresh water, with typically low concentration (mg/l) similar to average global values for river water of 4.1 (mg), 2.3 (K), 6.3 (Na), 15 (Ca) and

salt water, represented by average sea water levels (mg/l) of 1294 (Mg), 399 (K), 10770 (Na), 412 (Ca) (40), would cause dilution and enhancement, respectively, of these ions in vitreous humour. In cases of salt water drowning, Mg appears to offer the best prospect of yielding detectable changes in the vitreous humour, as shown from the figures obtained from the 2 cases of salt water drowning. To put the results on a more meaningful basis in the present study and also as a critical test of previously forwarded hypotheses (4), it might be helpful to follow the movement of magnesium and other ions from salt water to the aqueous and vitreous bodies. The eyeball, during immersion in sea water, has the aqueous portion in contact with this fluid and separated from it by the cornea. The inner layer of epithelium offers a high resistance to the movement of all ionic substances (30). However, when these ions are present in a large steady-state concentration, they will in time permeate the membrane and diffuse into the aqueous and vitreous humour. Further, there is an exchange between the aqueous and the vitreous humour. Exchange of ions across the boundary separating these two does not depend on active transport (68). Any net migration of substances in this direction depends largely on diffusion and will, in fact, diffuse throughout the aqueous and vitreous volumes at a rate close to that of H_2O. It has also been proved experimentally that the magnesium ions diffuse into the human eyeball after death when it is immersed in salt water (4). The same workers have demonstrated that the

rate of increase of magnesium concentration in the vitreous humour in relation to the time of immersion in hours is almost linear. This phenomenon is thought of special importance in considering the order of death among victims in a naval accident, but did not give absolute information about the time that the body was immersed in the water (4). Studies on both human (4) and bovine eyeballs (158) immersed in salt water, however, have shown that the establishment or exclusion of a diagnosis of drowning is not possible because of ion diffusion into the eyes of the immersed body, for which drowning need not have been the cause of death.

4.8. ESTIMATION OF IMMERSION TIME IN SALT WATER:

It was reported (158), that the water moves out of the eye into the surrounding hyperosmolar seawater, and magnesium and chloride ions move from the seawater into the vitreous humour.

Sturner et.al.(158) found a vitreous humour magnesium range of 21-97 mg/l. In the current study, magnesium values of 40.8 and 35 mg/l were recorded in the two cases of salt water drowning. Sodium also exceeded the control range while calcium was elevated with respect to the control mean value (Tab.3).

The estimated values of vitreous humour electrolytes in drowning cases were predicted (Figs.12-21) by using the regression equations of 24 h mean values of control data (Figs.7-11). Enhancements or decreases in the measured

values compared with the predicted ones show that the difference between the two values is a result of dilution during immersion, as it is seen as early as 5 h after immersion in fresh water. The potassium level exceeded that predicted on the basis of post-mortem interval. Chloride also showed very high level compared to that of control cases.

It has been postulated that the rate of diffusion of magnesium ions into vitreous humour from salt water is proportional to the time elapsed since death and that the vitreous humour magnesium concentration could be used to determine the time that a body has been immersed in salt water (4). Laboratory experiments with exenterated eyeballs have indeed shown that the wall of the eyeball acts as a permeable membrane through which water and ions can diffuse freely (158). In a surrounding salt water medium vitreous humour magnesium concentration were found to increase dramatically, e.g. to 340 mg/l in 24 h (4). Through comparable data from various experimental arrangements on eyeball immersion with levels found in human immersion fatalities it was shown that magnesium increased compared with the simulated experimental situation corresponding to the corneal exposure in an unstirred medium (158). However, the correlation of Mg with immersion time is not linear all the time, as there appear to be fluctuations, due to autolysis, total death interval until vitreous sampling and a variety of possible postmortem changes. These factors lead to a non-

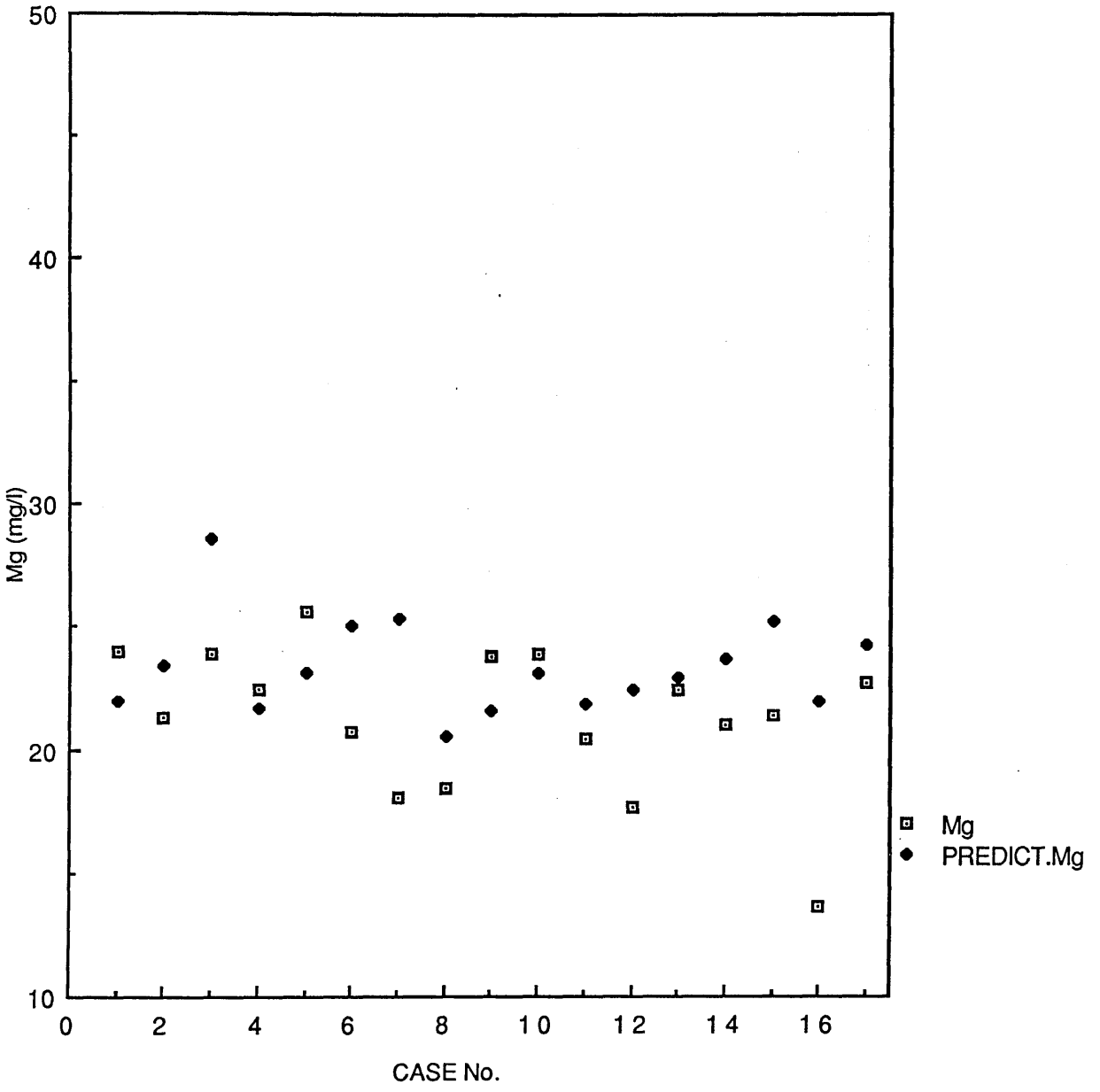

Fig. 12. Comparison of individual postmortem vitreous humour Mg levels (mg/l) for drowning victims , for the first 5 h immersion time, with predicted Mg values based on the linear regression equation in Fig. 7.

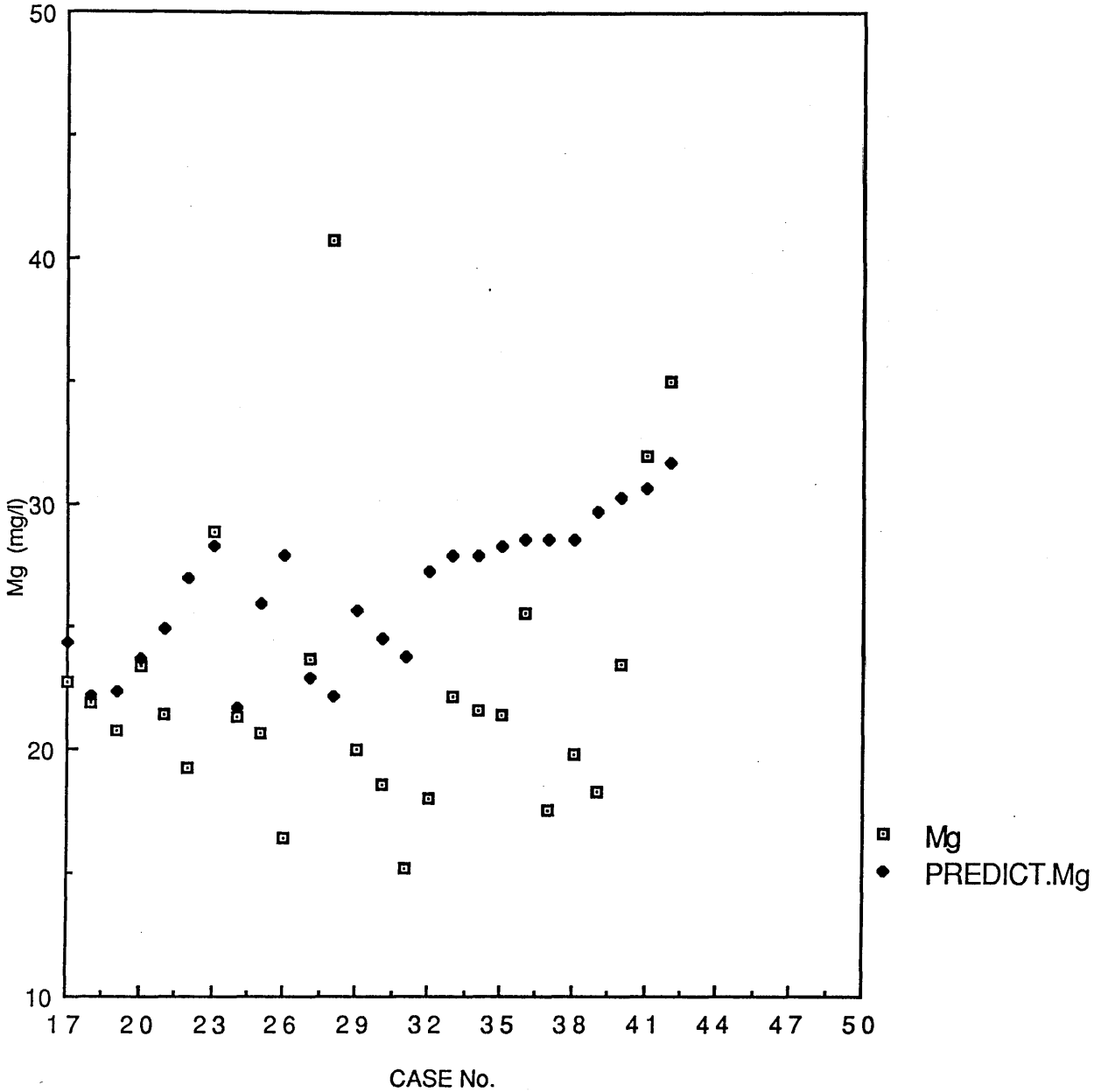

Fig. 13. Comparison of individual postmortem vitreous humour Mg levels (mg/l) for drowning victims , for more than 5 h immersion time, with predicted Mg values based on the linear regression equation in Fig. 7.

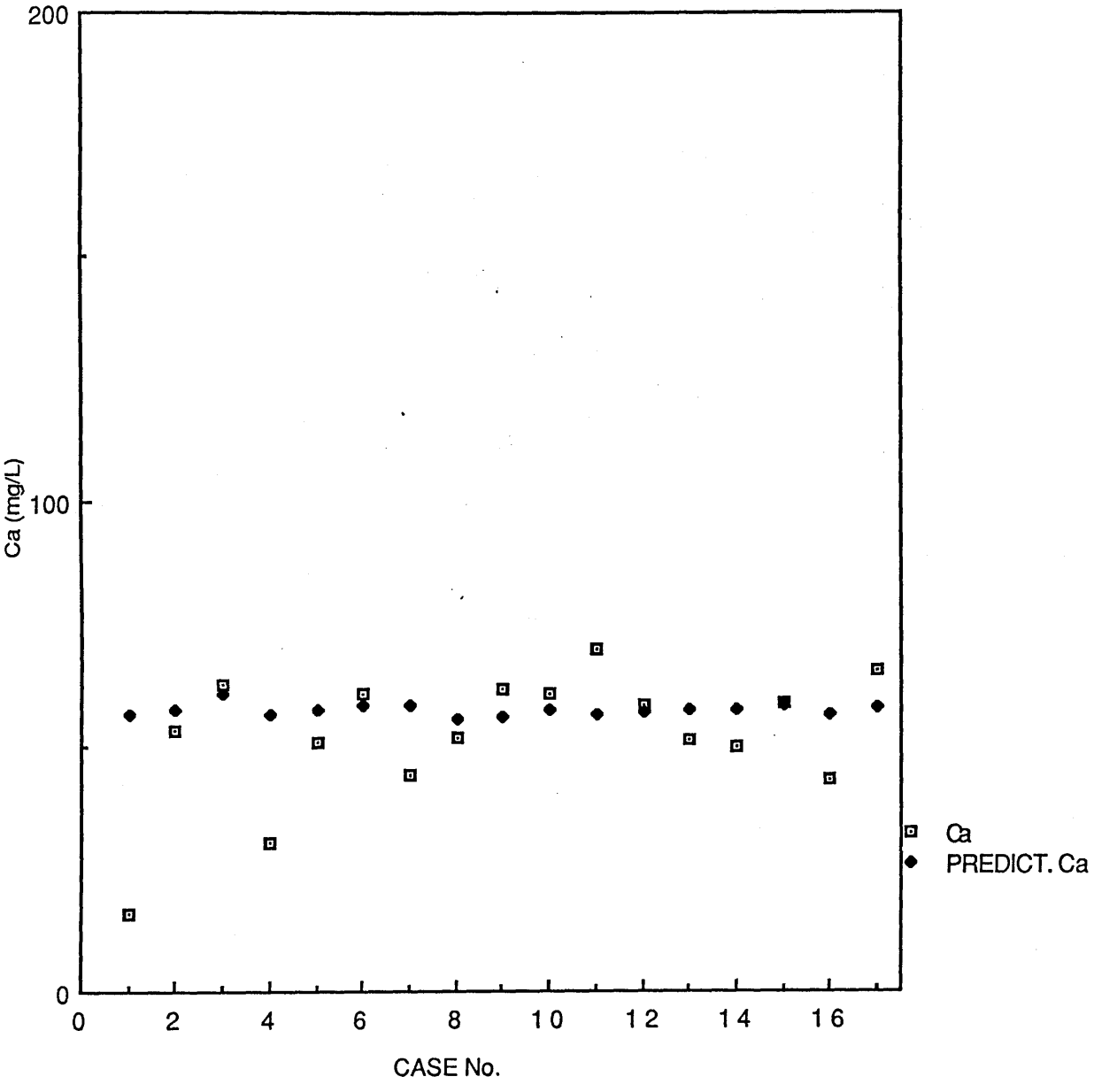

Fig. 14. Comparison of individual postmortem vitreous humour Ca levels (mg/l) for drowning victims , for the first 5 h immersion time, with predicted Ca values based on the linear regression equation in Fig. 8.

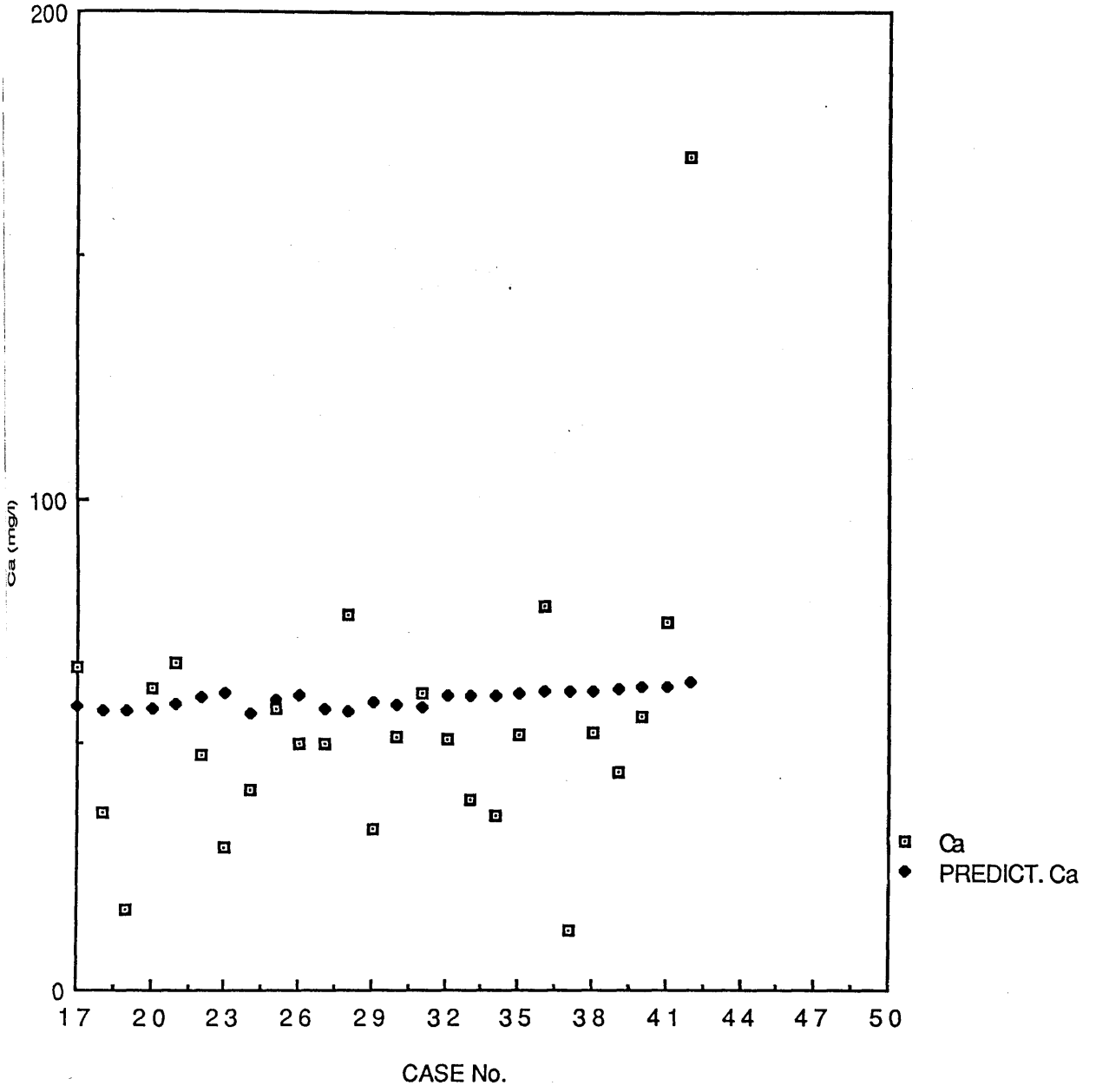

Fig. 15. Comparison of individual postmortem vitreous humour Ca levels (mg/l) for drowning victims , for more than 5 h immersion time, with predicted Ca values based on the linear regression equation in Fig. 8.

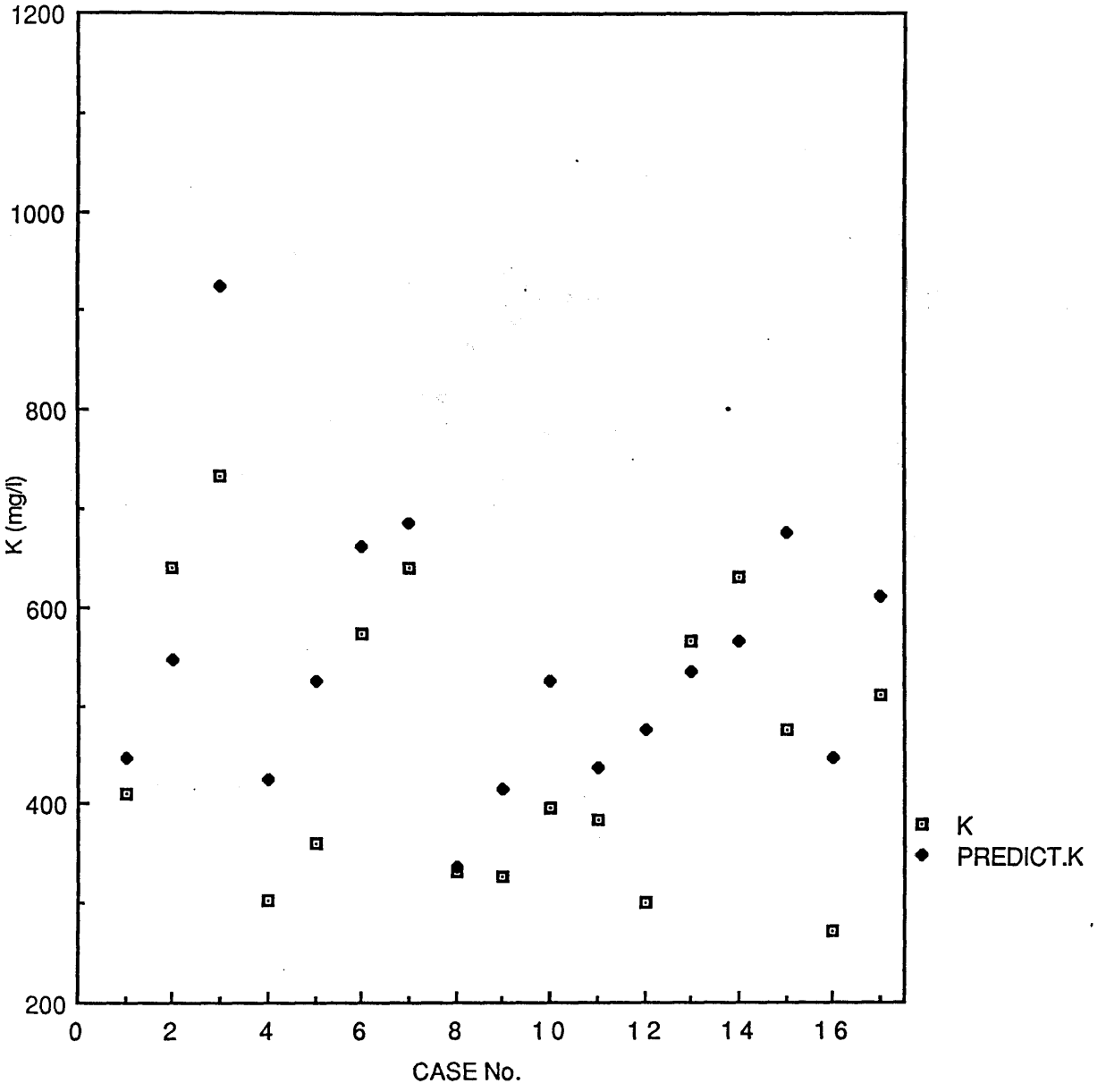

Fig. 16. Comparison of individual postmortem vitreous humour K levels (mg/l) for drowning victims , for the first 5 h immersion time, with predicted K values based on the linear regression equation in Fig. 9.

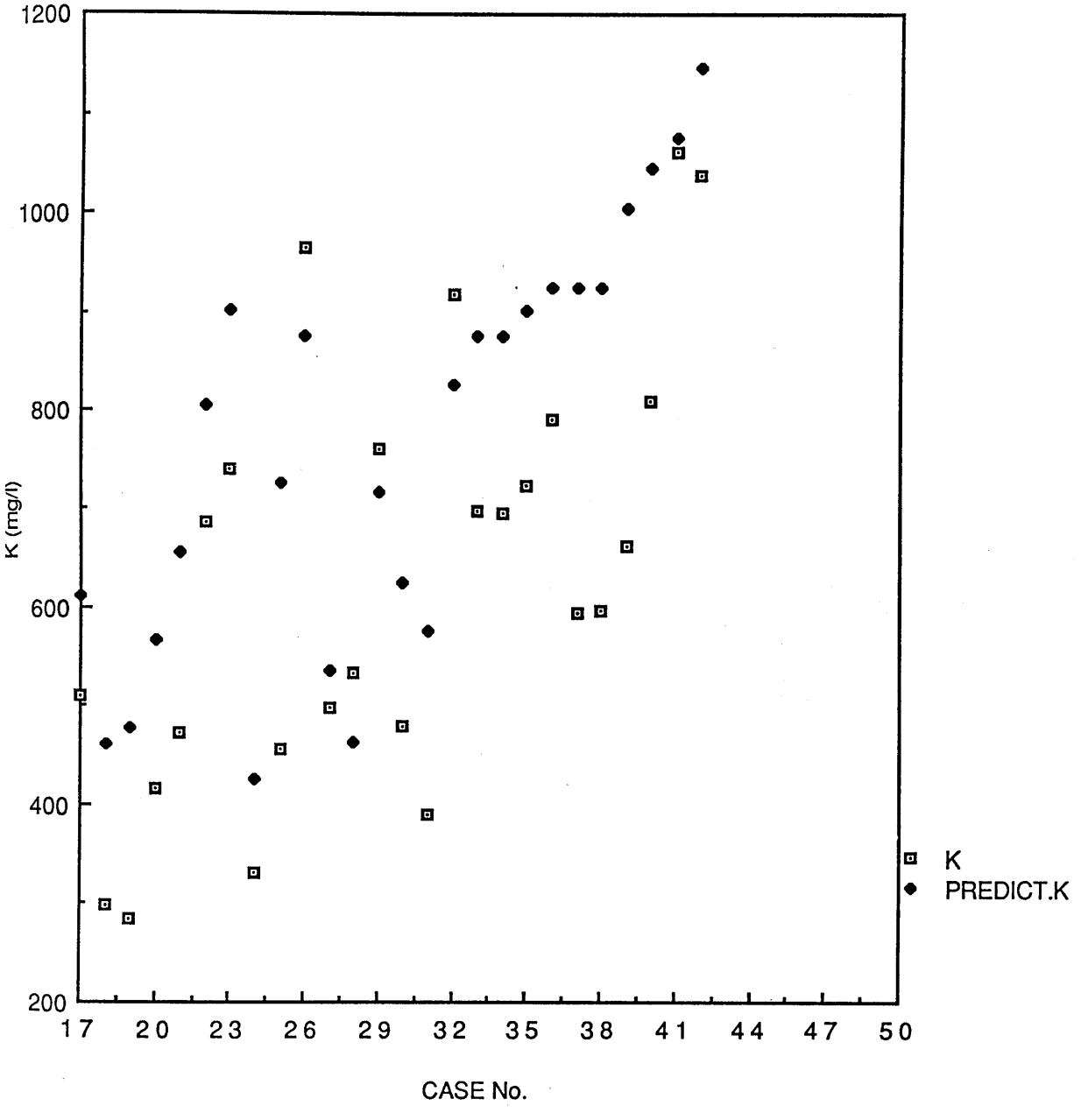

Fig. 17. Comparison of individual postmortem vitreous humour K levels (mg/l) for drowning victims , for more than 5 h immersion time, with predicted K values based on the linear regression equation in Fig. 9.

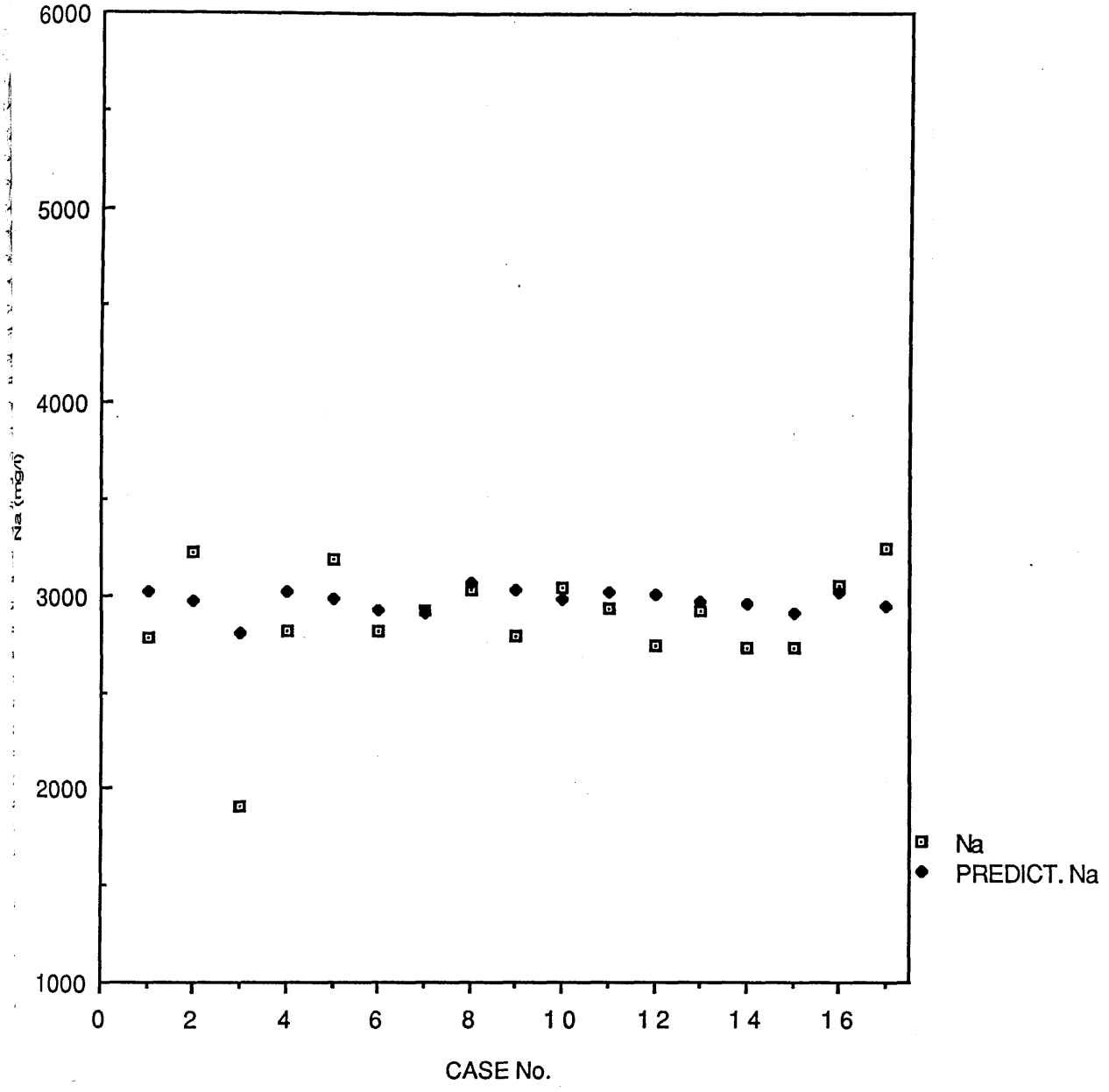

Fig. 18. Comparison of individual postmortem vitreous humour Na levels (mg/l) for drowning victims , for the first 5 h immersion time, with predicted Na values based on the linear regression equation in Fig. 10.

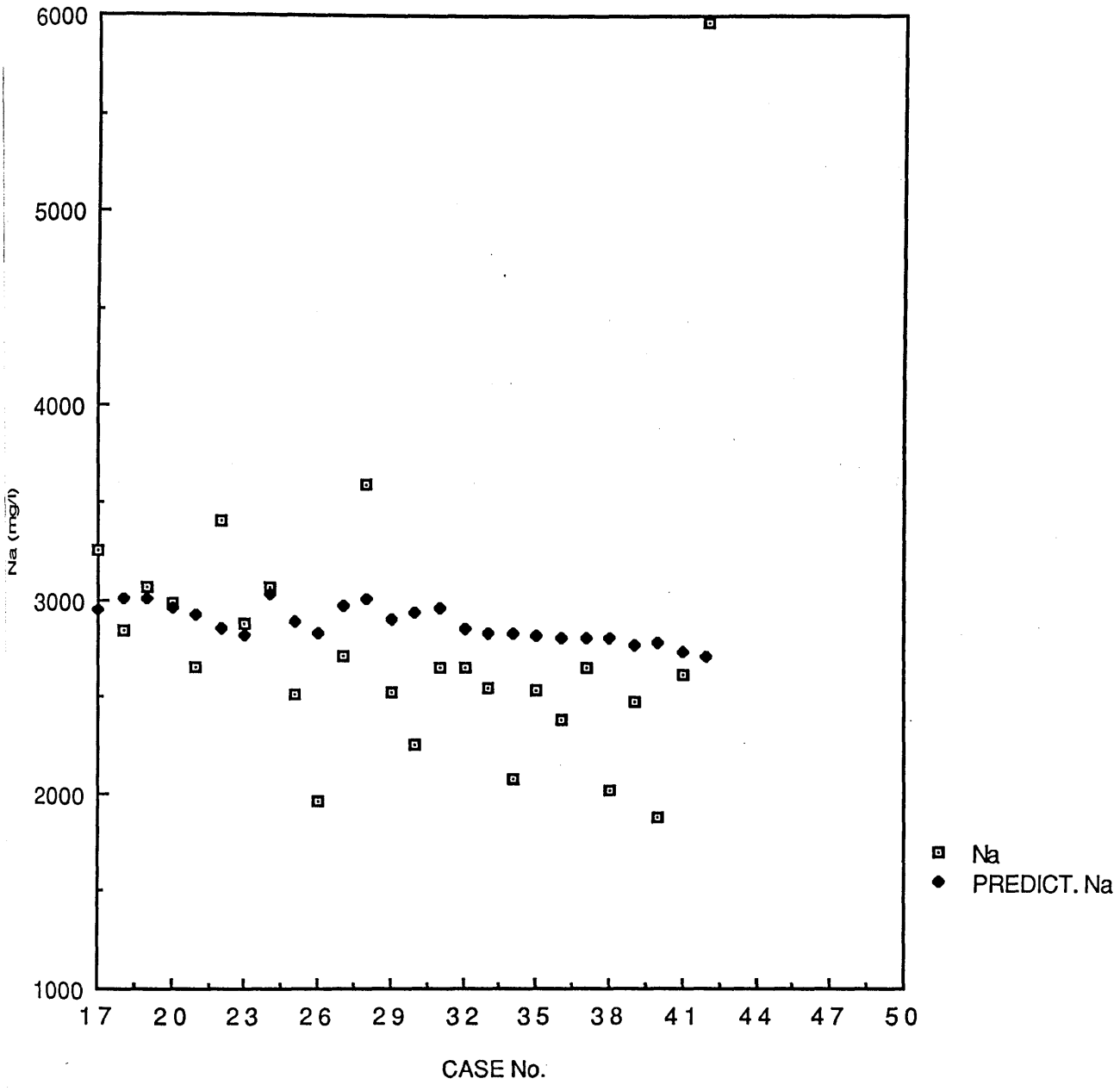

Fig. 19. Comparison of individual postmortem vitreous humour Na levels (mg/l) for drowning victims , for more than 5 h immersion time, with predicted Na values based on the linear regression equation in Fig. 10.

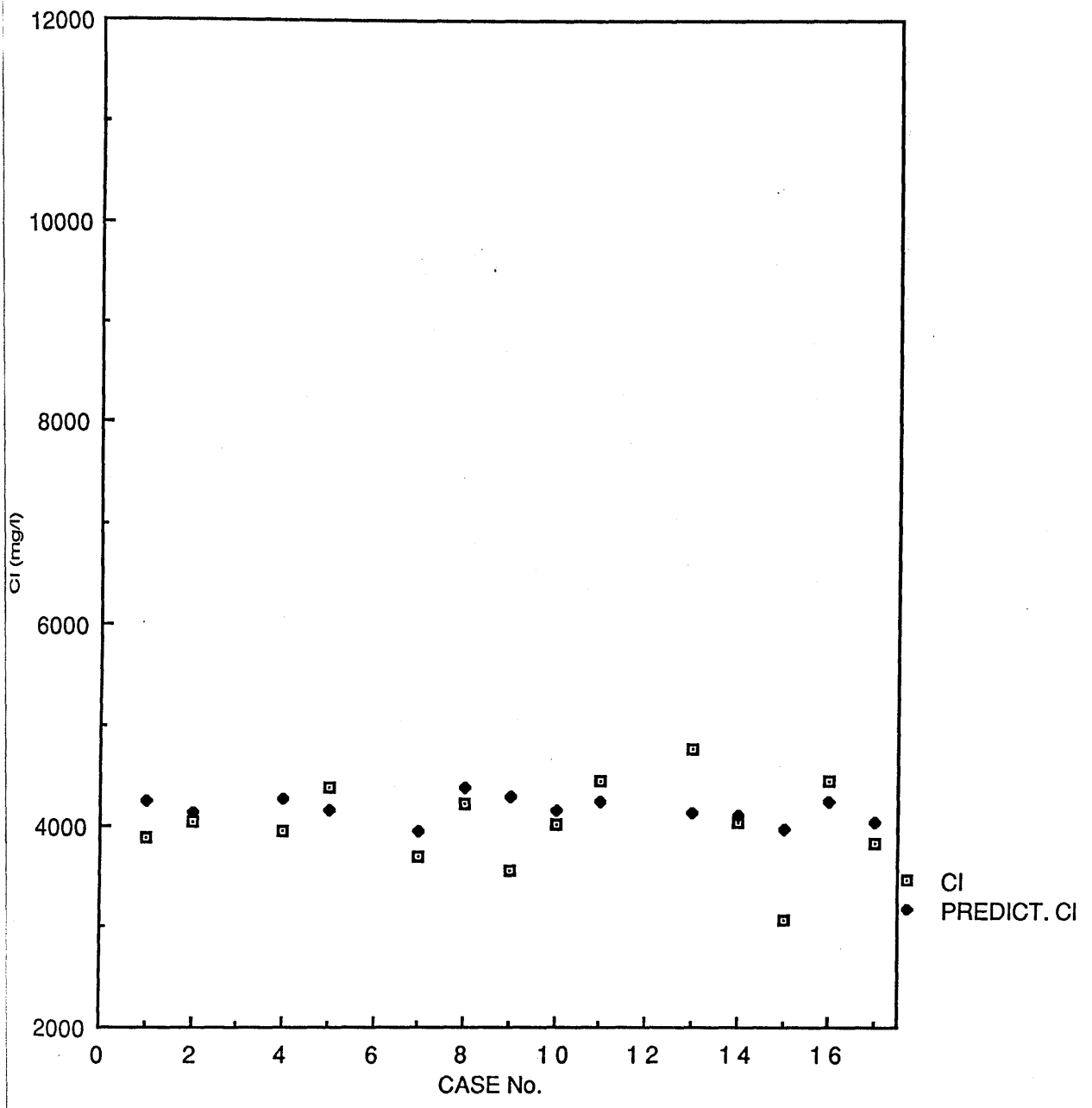

Fig. 20. Comparison of individual postmortem vitreous humour Cl levels (mg/l) for drowning victims , for the first 5 h immersion time, with predicted Cl values based on the linear regression equation in Fig. 11.

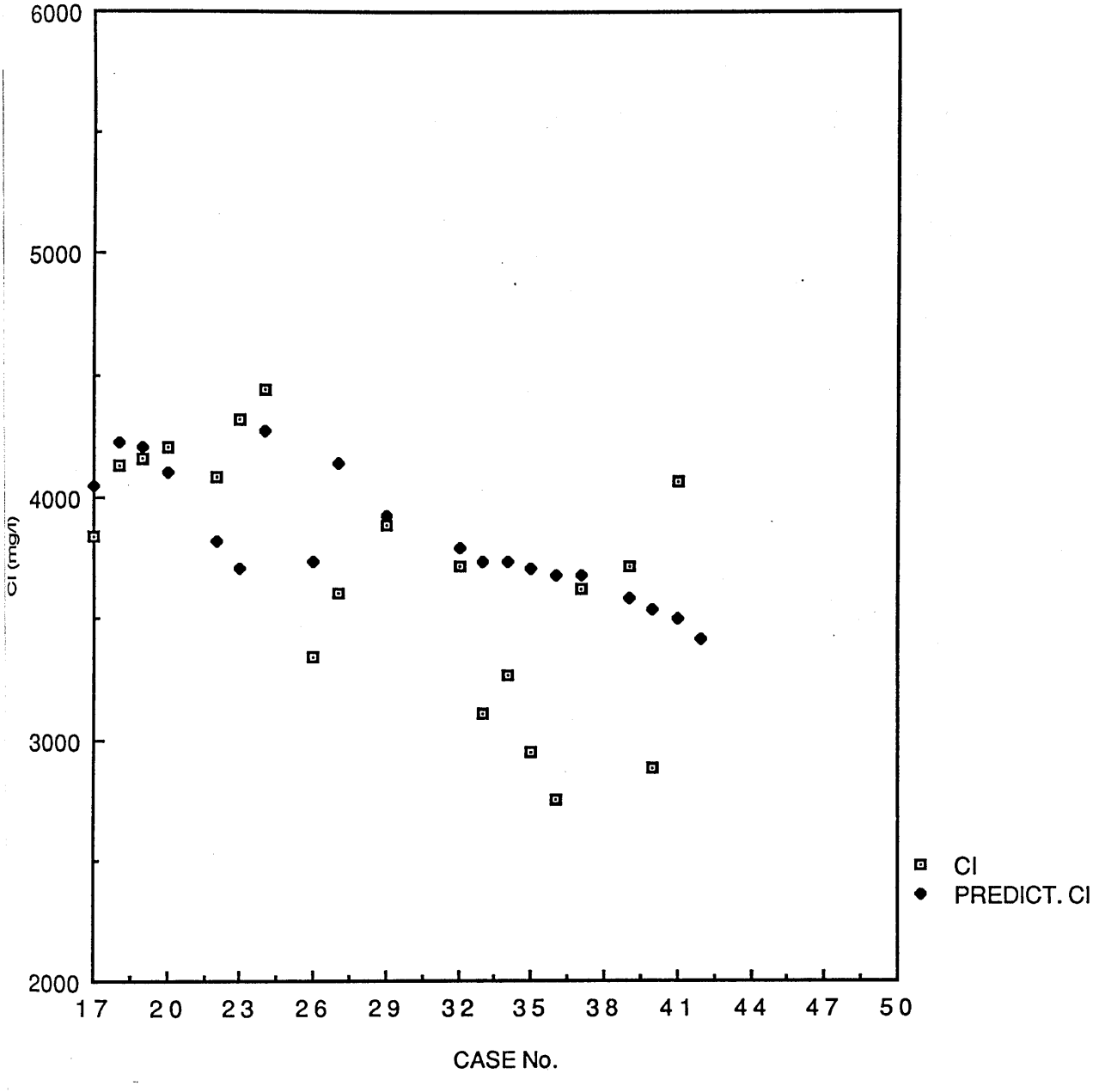

Fig. 21. Comparison of individual postmortem vitreous humour Cl levels (mg/l) for drowning victims , for more than 5 h immersion time, with predicted Cl values based on the linear regression equation in Fig. 11.

linear and irregular relationship between magnesium and immersion time.

4.9. ESTIMATION OF IMMERSION TIME IN FRESH WATER

It was possible to make some observations based on the two sets of results obtained from drowning and control cases. The first was that the vitreous humour Mg, Ca, K, Na and Cl concentration was significantly higher in the control cases than in drowning cases. The second is that the dilution of vitreous electrolytes in fresh water submersion may be caused both by water entering the hyperosmolar vitreous humour and ions diffusing out from it. From the results of Mg, Ca, Na, K and Cl of 41 fresh water drowning cases, fresh water immersion result in dilution of vitreous humour ion concentration relative to control cases (Tab.3 and Figs.12-21). The electrolyte concentration in the vitreous humour of drowning victims will be subject to influence both by postmortem interval and the immersion time. In drowning cases, the predicted values of Mg, Ca,K, Na, and Cl based on the length of postmortem interval were calculated from the regression equations obtained from the mean measured values of these elements in the vitreous humour, as shown in the Figs.(7-11). As potassium increases markedly with postmortem interval, any comparison of measured control and drowning K data would be particularly meaningless. In 35 cases out of 41 cases of fresh water drownings there is a reduction in the values of K which is significant in 26 cases, of 100-350 mg/l. (Figs.16,17 & 24). The difference between

the measured and predicted values, represented by delta (Δ), have been expressed as percentage of the predicted K, and showed that in 38 cases there is an average reduction of more than 10% in the predicted K concentration (Fig.30).

The relationship with immersion time does not appear to be simple and, as with the increase in magnesium in salt water drownings, a variety of post-mortem and environmental influences may account for individual variation in fresh-water dilution. As shown from Fig.(28) there is no consistent relationship between the dilution of measured values of Mg with the immersion time. This might be due to individual variation in the rate of dilution of vitreous humour by fresh water.

In nearly 63% of fresh water drowning cases there is a significant reduction of the measured values of Na, as compared with the predicted values (Fig.18,19 & 25). The predicted values are obtained from the equation in Fig.(10). However the degree of depletion of Na cannot be related to the time of immersion, as there is no consistent relationship between the percentage of ΔNa, which is expressed as % of predicted Na values, and the immersion time (Fig.31). Calcium also showed the same degree of dilution with a degree of depletion of not more than 50% of the predicted values (Figs.14,15,23 &29). Since, the rate of depletion of Ca is not predictable, as the same percentages of depletion were detected at different immersion times, Ca is, also, disregarded as an

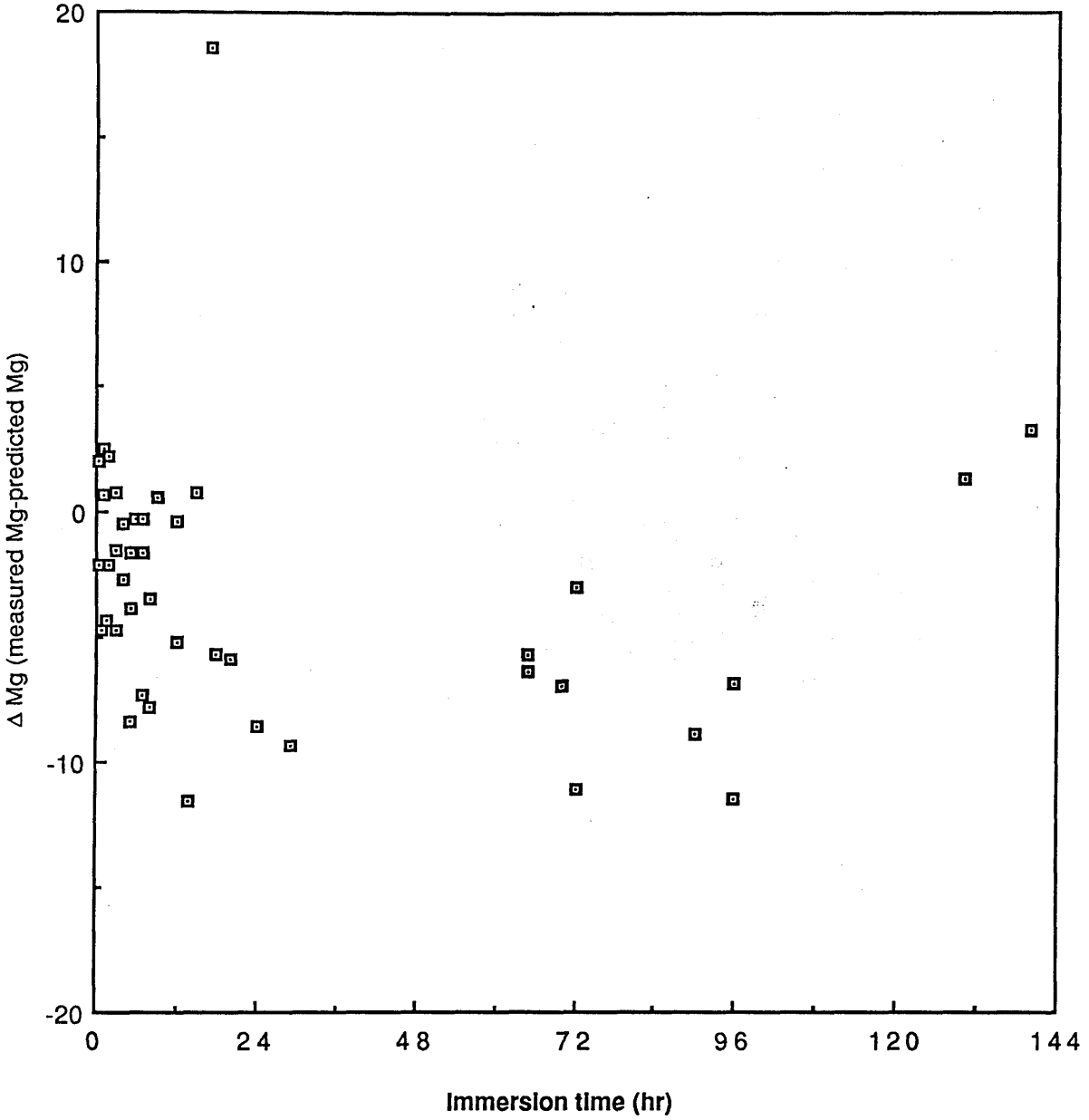

Fig. 22. Difference in the concentration of measured and predicted values of Mg (Δ Mg) for drowning victims plotted against the immersion time.

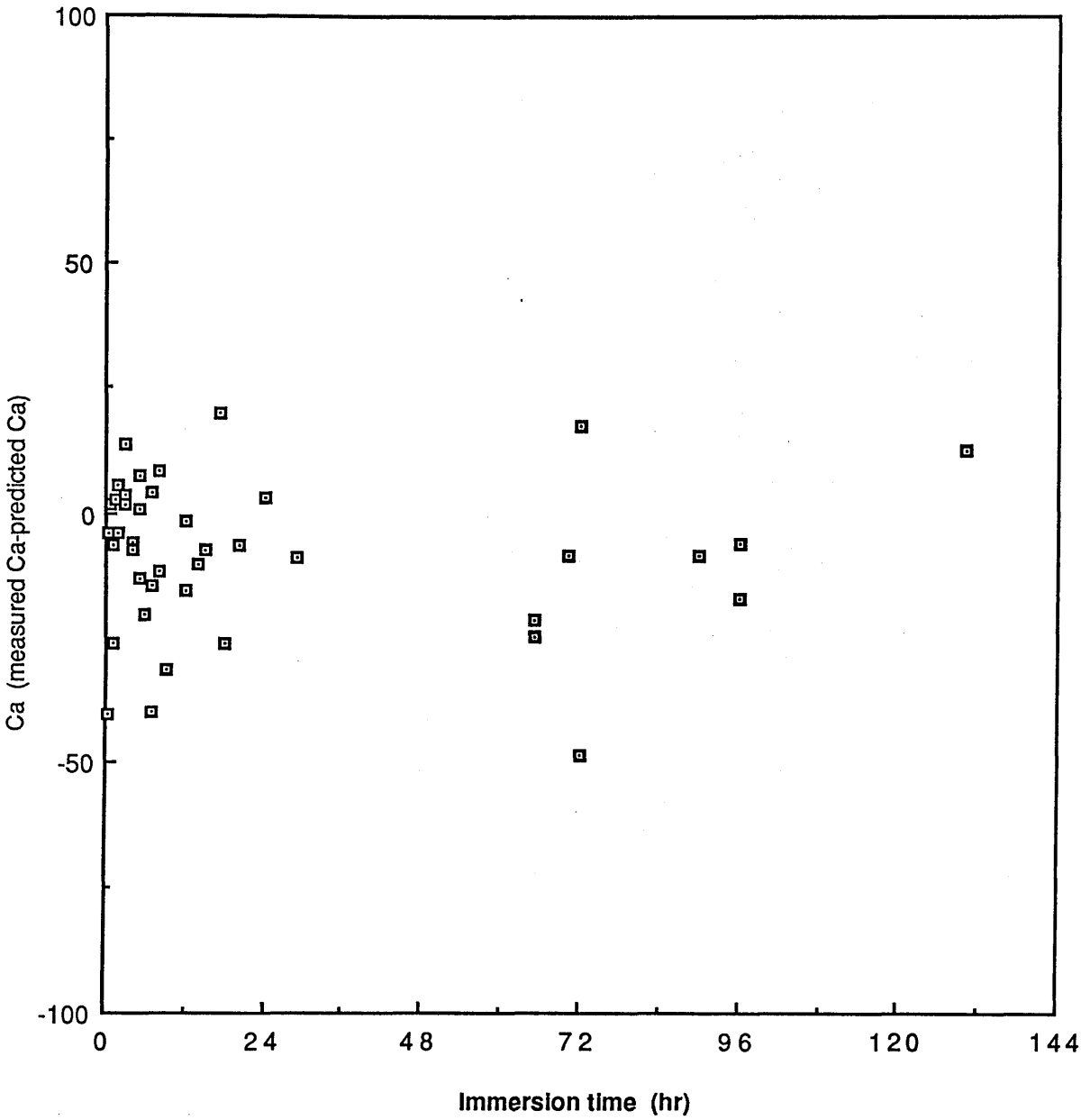

Fig. 23. Difference in the concentration of measured and predicted values of Ca (Δ Ca) for drowning victims plotted against the immersion time.

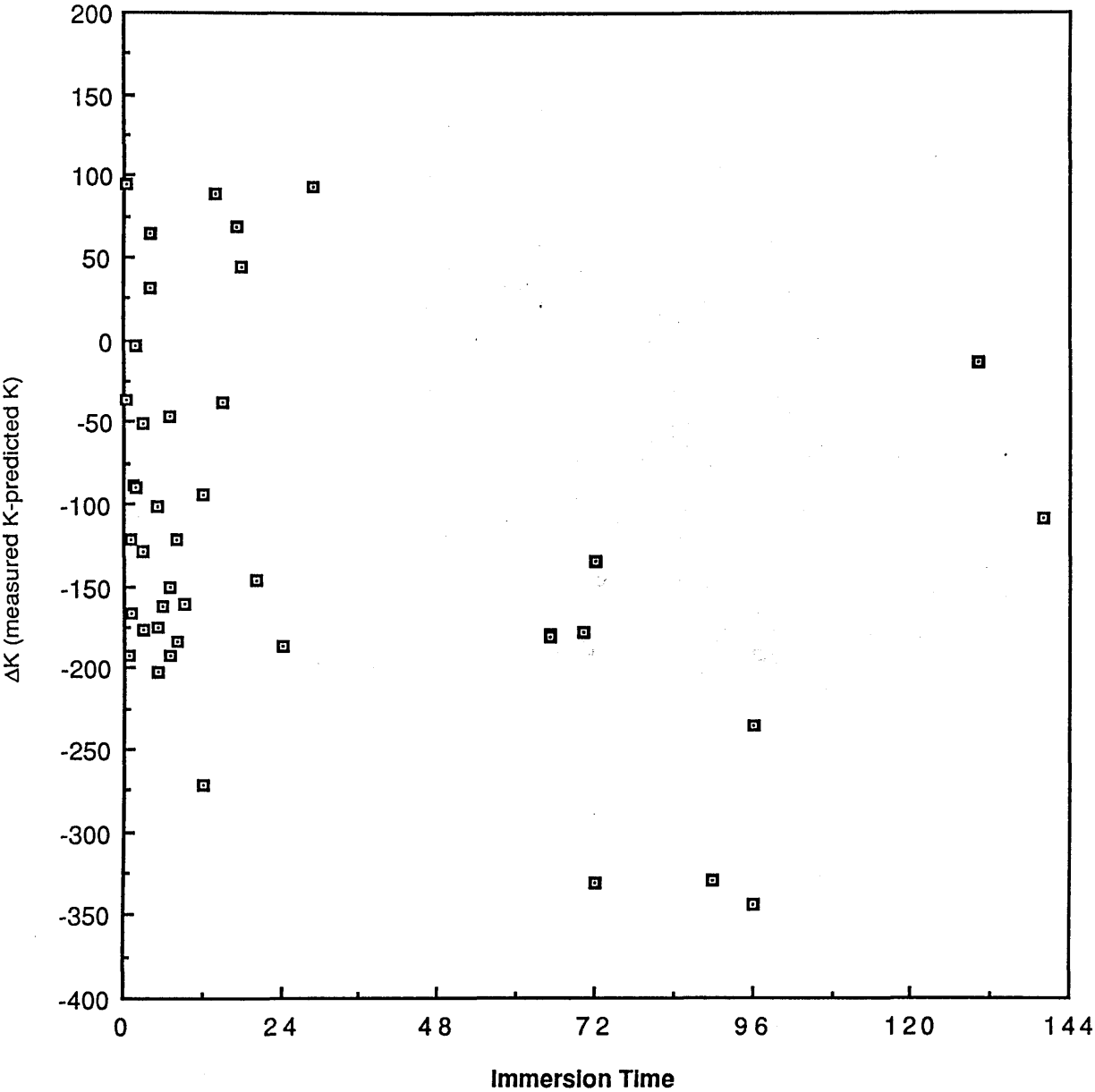

Fig. 24. Difference in the concentration of measured and predicted values of K (Δ k) for drowning victims plotted against the immersion time.

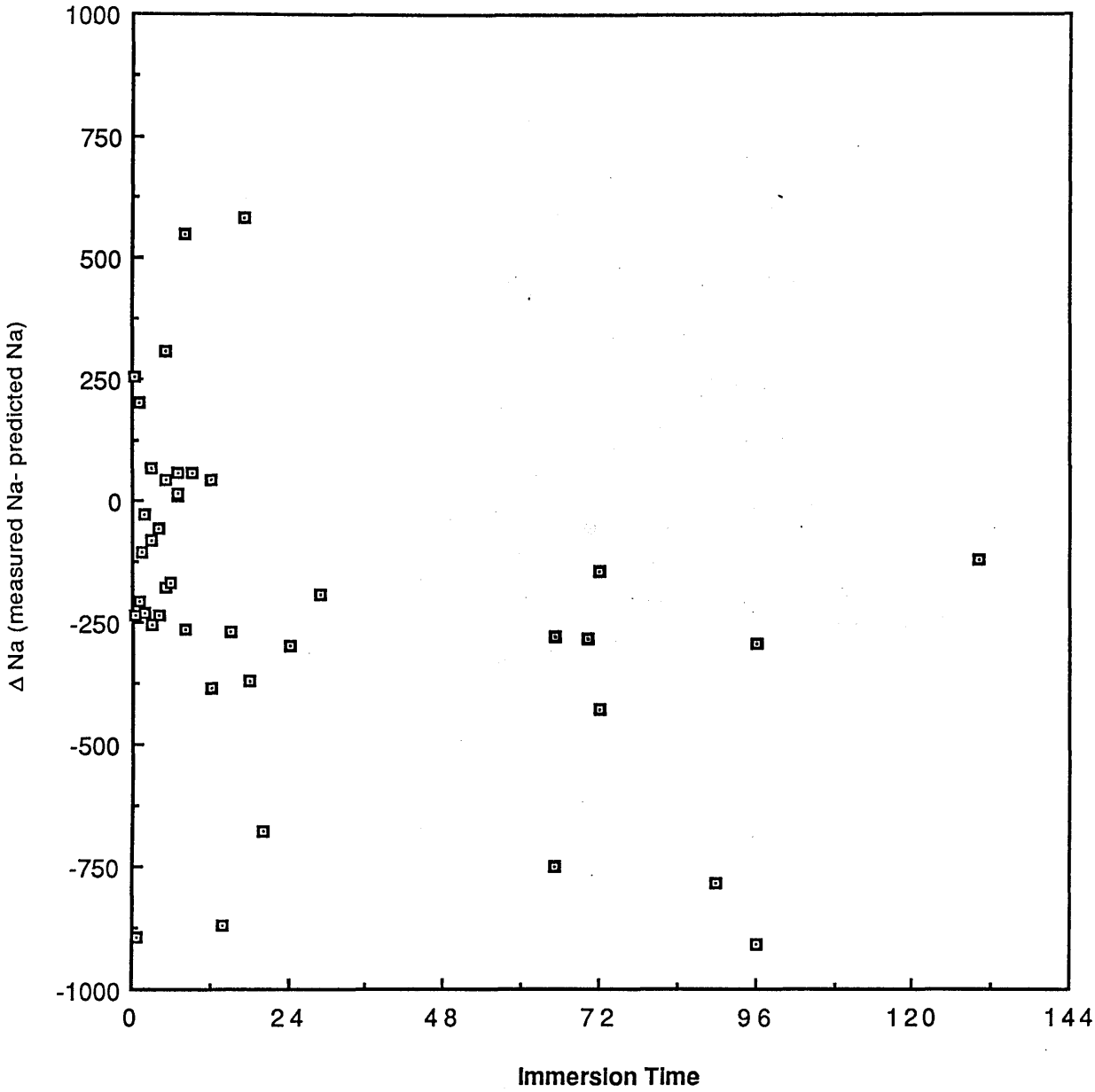

Fig. 25. Difference in the concentration of measured and predicted values of Na (Δ Na) for drowning victims plotted against the immersion time.

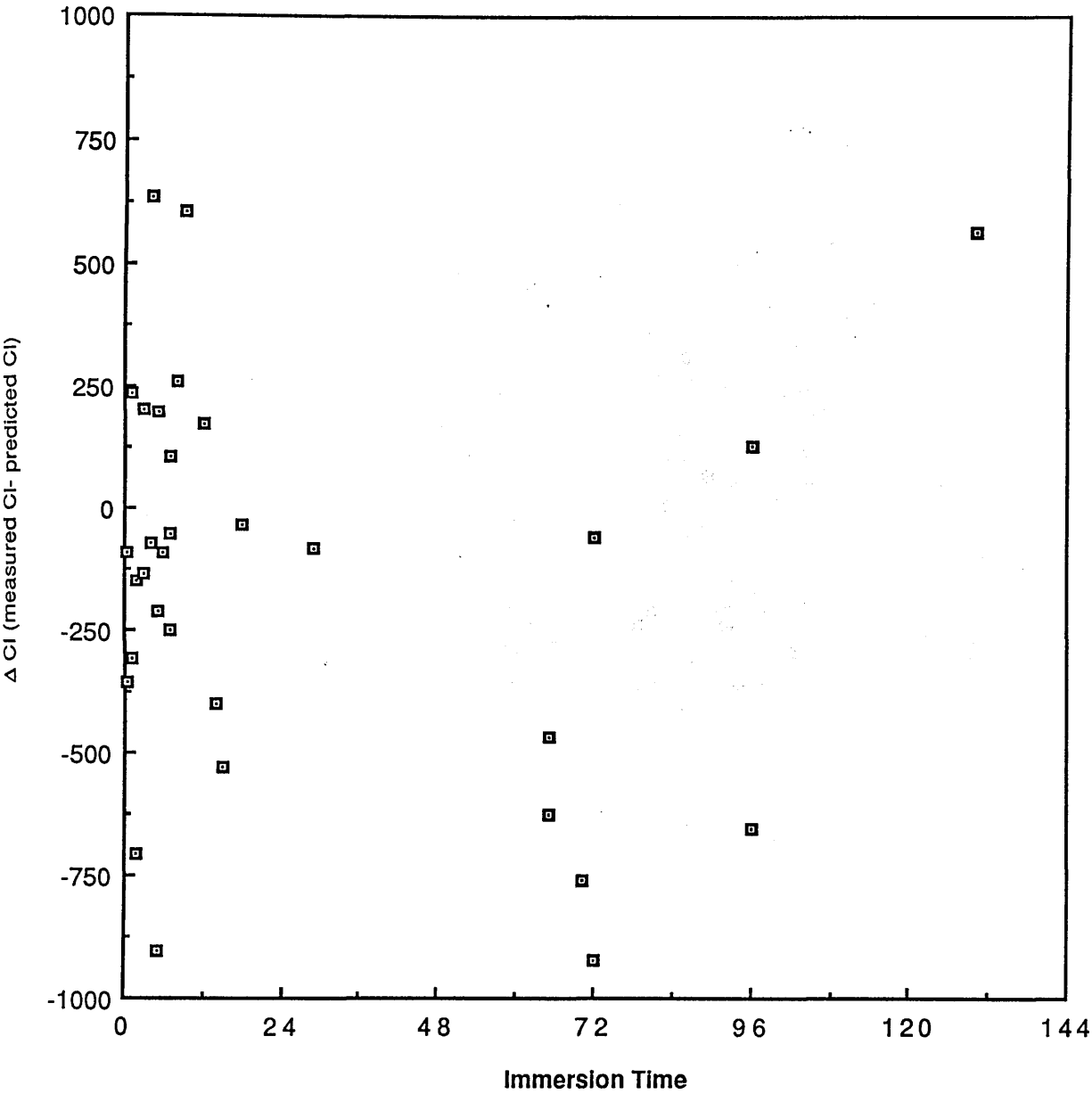

Fig. 26. Difference in the concentration of measured and predicted values of Cl (Δ Cl) for drowning victims plotted against the immersion time.

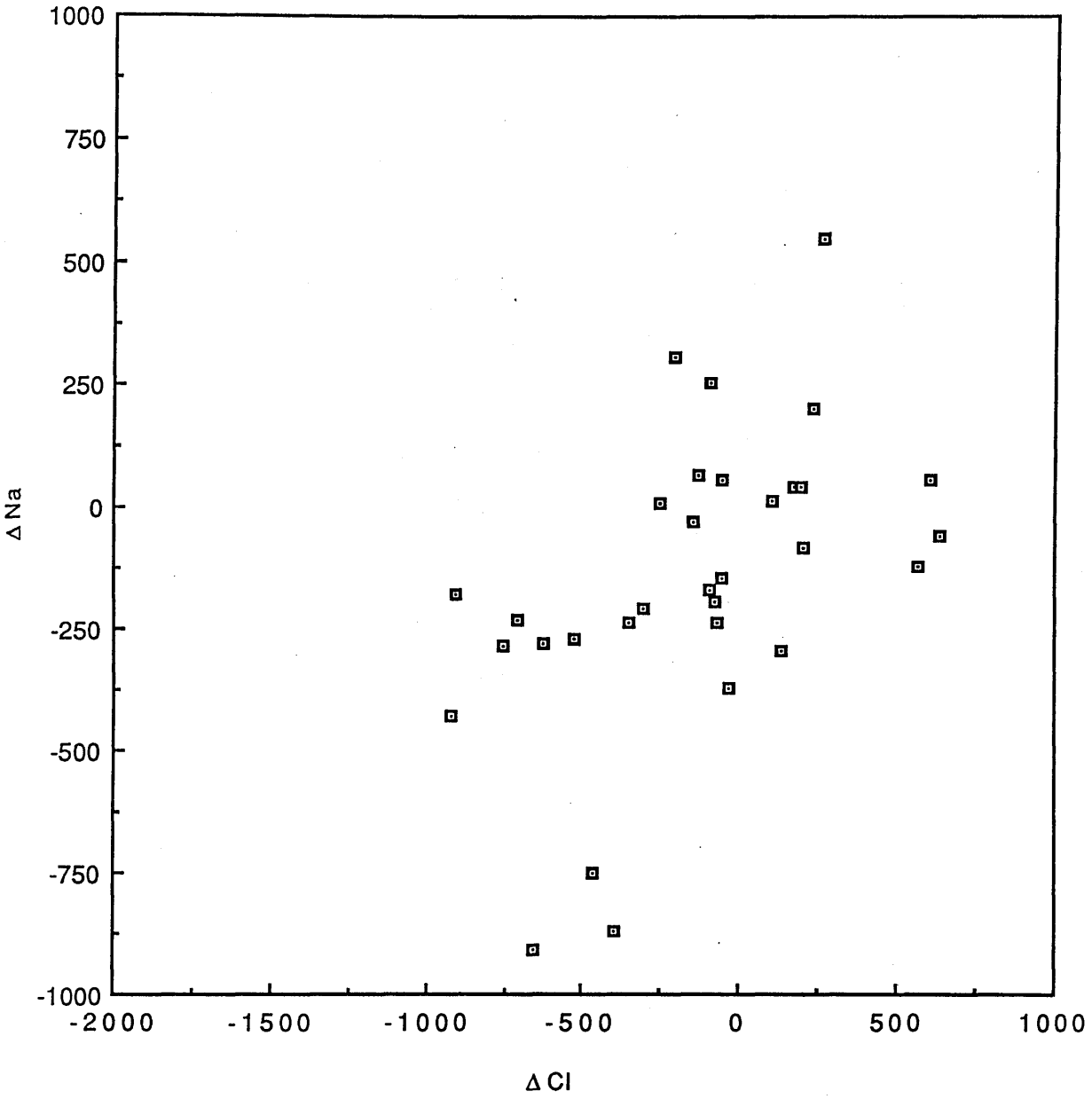

Fig. 27. (Δ Na) plotted against (ΔCl).

indicator of vitreous humour dilution in fresh water drowning.

Chloride, although it has shown a higher percentage of dilution (Fig.26) compared to both Sodium and Calcium, still exhibits a similar degree of depletion irrespective of the length of immersion time. Also there is inconsistent relationship between the degree of dilution and the immersion time, as similar percentages of depletion were detected at different periods of immersion (Fig.26 and 32). With the other two elements, i.e. magnesium and potassium, dilution has been observed in nearly 75% of the cases (Figs.12 & 13 for Mg) and (16 & 17 for K). The rate of depletion showed the same phenomenon, as the other electrolytes of vitreous humour, of inconsistency with the time of immersion. However there is an obvious decline in the concentration of these elements with increasing time of immersion (Fig.22(Mg) and Fig.24(k)). In cases of fresh water drownings, there is a significant reduction in the amount of measured values of Mg, Na, Ca, K and Cl compared with the predicted values. In a few fresh water drowning cases there is an enhancement of the measured values of Mg, Ca, Na, K and Cl over the predicted values of these elements. These values are not related to any particular time of immersion, i.e. they are not uniformly altered at a definite time.

Dilution of sodium in vitreous humour in fresh-water drowning victims was observed by Bray et.al.(15) who

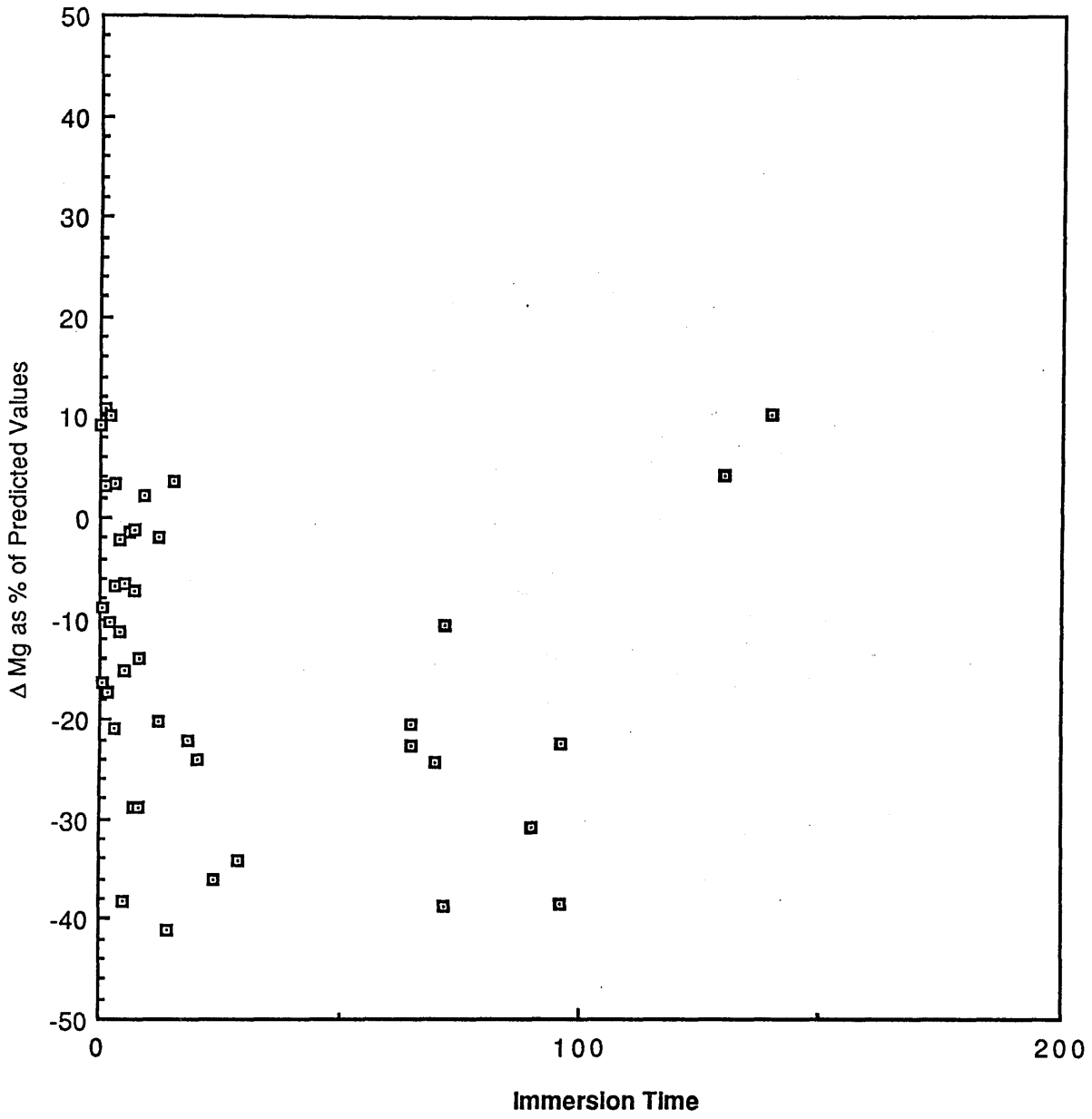

Fig. 28. The percentage of dilution of Mg (ΔMg) expressed as percentage of the predicted values.

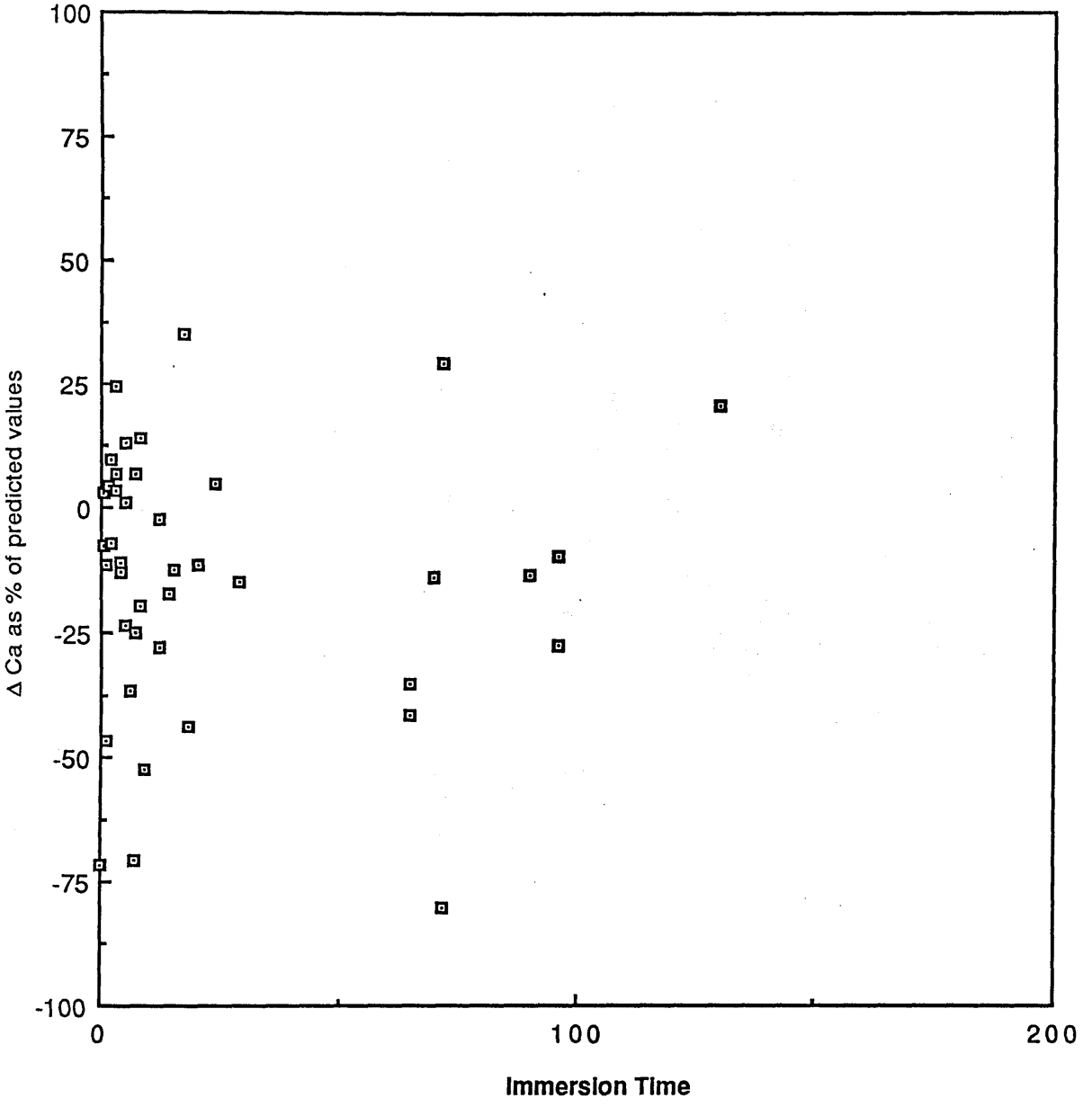

Fig. 29. The percentage of dilution of Ca (ΔCa) expressed as percentage of the predicted values.

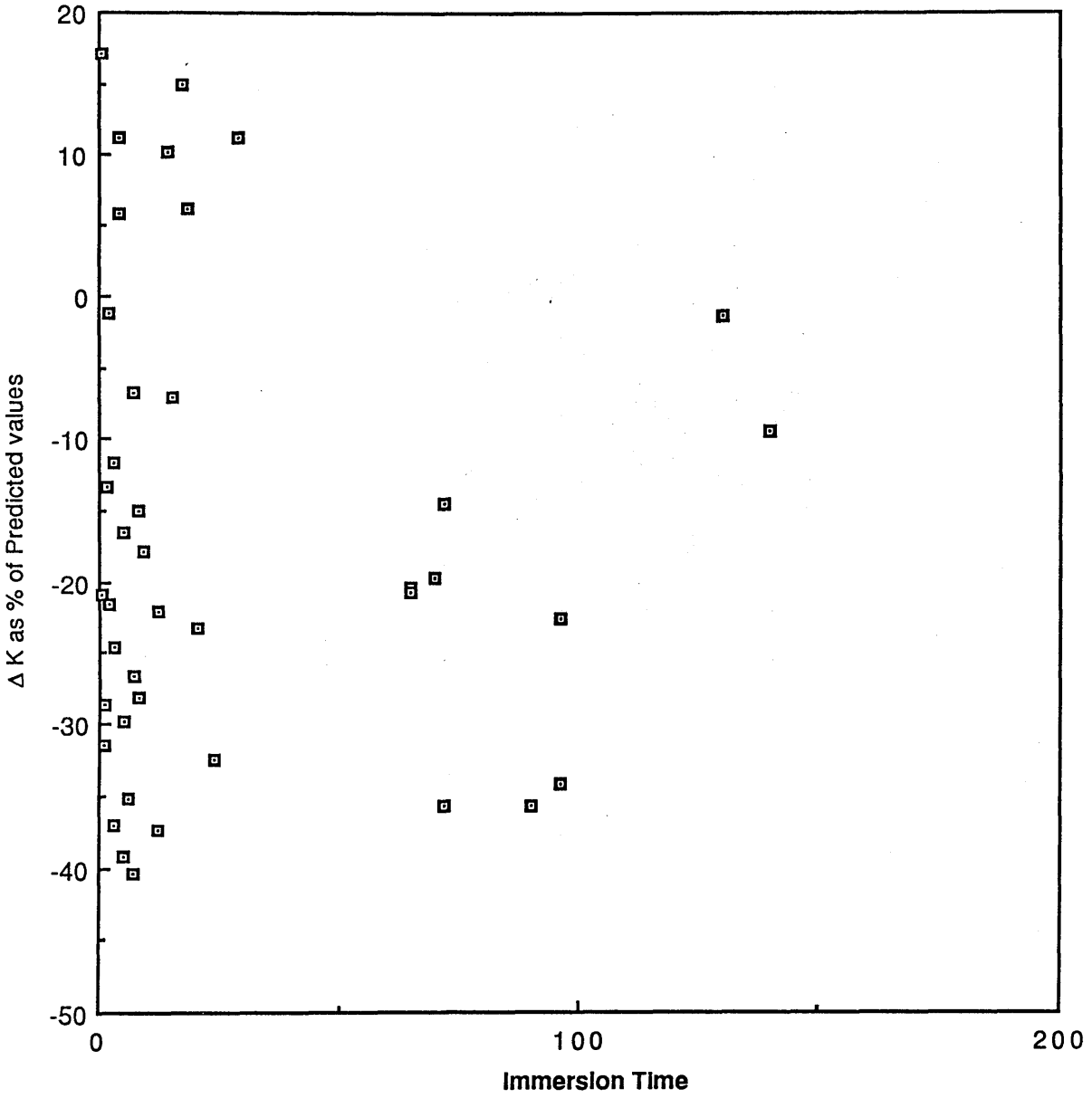

Fig. 30. The percentage of dilution of K (ΔK) expressed as percentage of the predicted values.

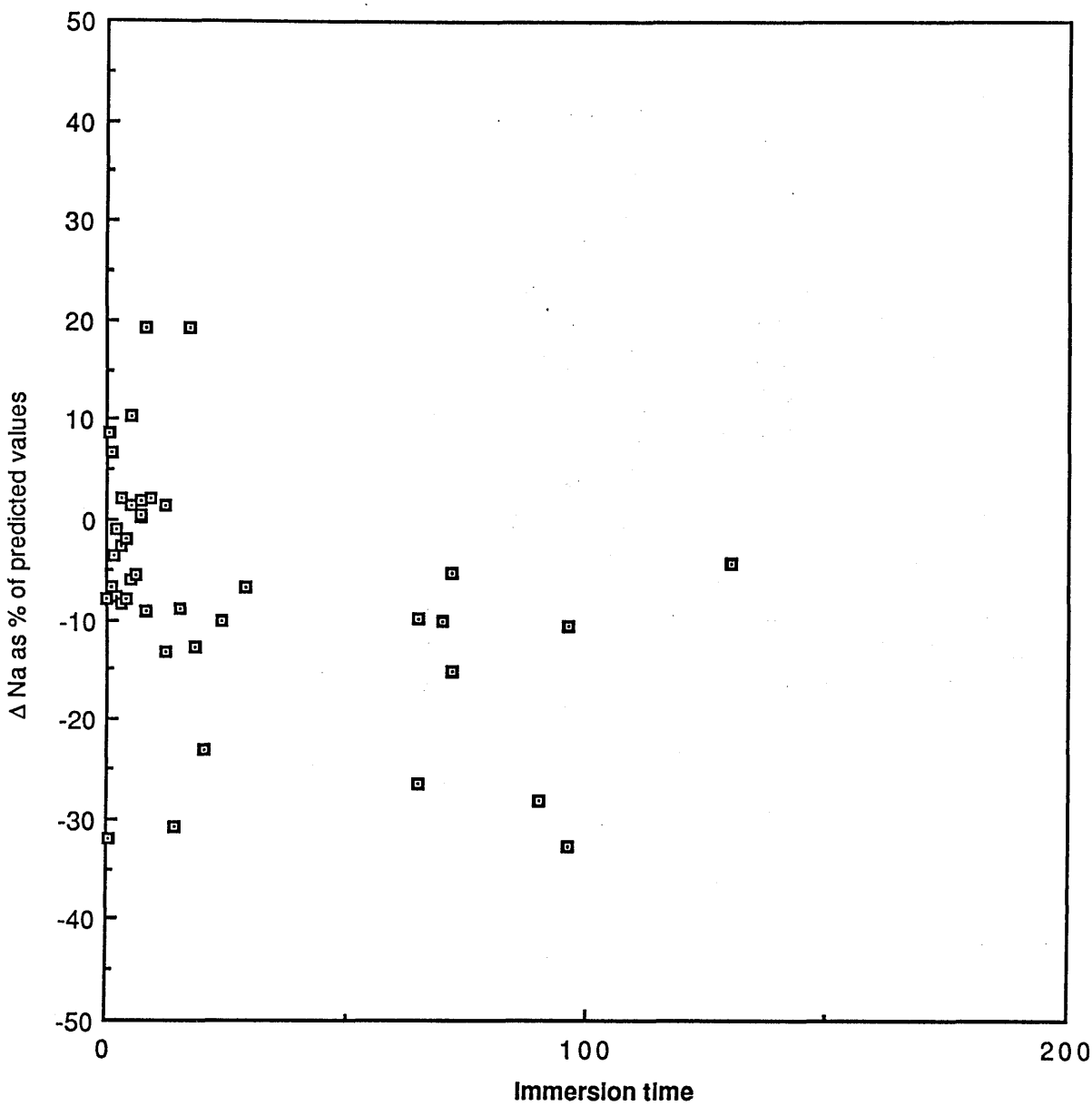

Fig. 31. The percentage of dilution of Na (ΔNa) expressed as percentage of the predicted values.

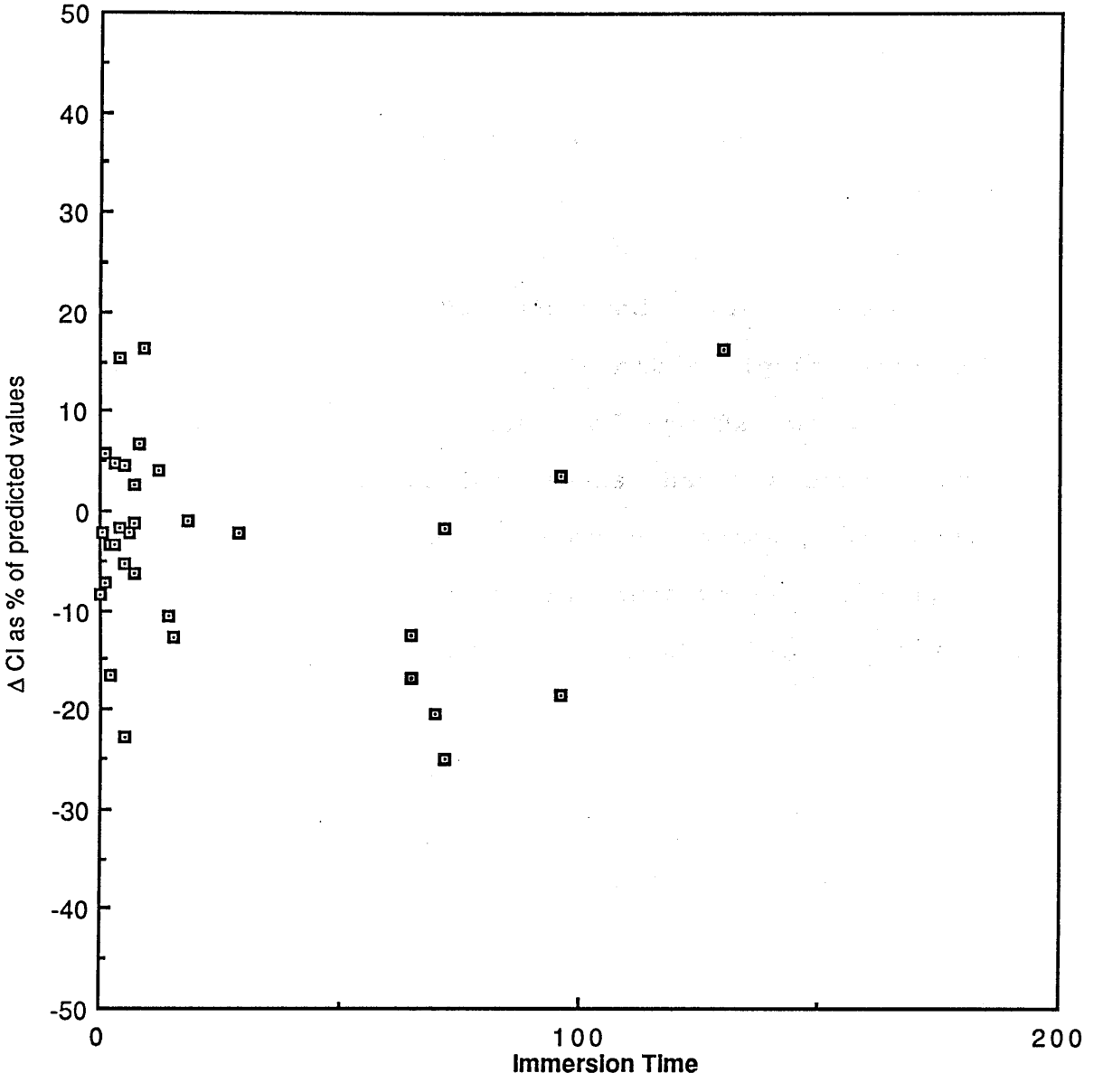

Fig. 32. The percentage of dilution of Cl (ΔCl) expressed as percentage of the predicted values.

found a range of 1725-2415 mg/l(mean 2192 mg/l) for sodium in 13 plane crash victims who had been immersed in fresh water for 7-8 days. It was also inferred from a comparison of the trends in sodium, chloride and potassium values that potassium was also affected by dilution with fresh water, though to a variable extent. The relation of Δ Na (measured value - predicted value) with that of Δ Cl is sufficiently significant to show a correlation coefficient of +0.92 between the two variables (Fig.27). This means that the concentrations of sodium and chloride in vitreous humour, are diluted to the same relative extent in fresh water drownings. Sodium and chloride, on account of the large concentration differences between vitreous humour and fresh water and a low rate of natural post-mortem change (Fig.10), are probably promising elements for investigation in fresh-water drownings. In this study, there is a highly significant difference between the mean sodium and chloride data for drowning victims and the controls (Table 3). The extent of the dilution of each of 41 cases of fresh water drowning victims is shown by the disparity between observed and predicted values (Figs.12-21 and 22-26).

5. CONCLUSION :

Chemical determinations on post-mortem biological fluids can be of value in estimating the time since death. The vitreous of the eye shows distinct advantages in terms of isolation from rapid changes in the state of

the body as well as ease of availability. The vitreous potassium level offers a more accurate measure than other constituents of the intraocular fluid in this endeavor, but its application has so far been sporadic in most laboratories.

Attempts to define the post-mortem interval have not meet with much success in the past. Unfortunately, little is known about the normal values of the various biochemical parameters in the human vitreous and aqueous humour. Accordingly, levels of magnesium, calcium, potassium, sodium, and chloride in the post-mortem vitreous humour from controls and drowning victims have been determined. The aim of the study was to find an accurate way of estimating the immersion time i.e. the time elapsed from the moment of immersion to the time of recovery of the body from water. A technique for estimation of post-mortem interval in other causes of death without immersion was followed. The effects of death-related internal changes, external environmental parameters and different causes of death were carefully assessed.

A positive correlation between potassium levels and the post-mortem interval has been established. Other vitreous electrolytes elements, Mg, Na, and Cl have shown a relatively significant correlation, but to a lesser extent than potassium. At best, there is only a 2/3 chance of the prediction of post-mortem interval being within nearly 9 hours of the true value. The results of

this study reaffirm the arithmetic linear relationship of increase in vitreous potassium concentration with lengthening of the post-mortem interval up to 115 hours, which was the highest limit included in this study.

The individual biological variability limits the usefulness of predictions of post-mortem interval based on electrolyte ion data. It was not expected that vitreous humour chemistry studies would fully clarify the extent to which drowning was a main cause or contributory factor in causing death during immersion in water. No correlation was observed between the degree of pulmonary oedema and congestion, common anatomic correlates of drowning, and the concentration of any of the solutes measured in our investigation.

This study demonstrated dilution of vitreous humour electrolytes in the course of fresh water immersion. It appears that dilution of these in fresh water submersion may be caused both by entering the hyperosmolar vitreous humour and ions diffusing out of it. Individual variation in the degree of dilution should be kept in mind in attempting to interpret vitreous humour chemistry results in cases of prolonged submersion. In cases where drowning is suspected, establishment or exclusion of this cause of death is not possible on the basis of vitreous humour electrolyte data because of possible post-mortem diffusion across the permeable membrane of the eyeball.

The accurate estimation of the time of immersion in water according to the phenomenon of vitreous humour

dilution was not possible, although there was an obvious depletion of vitreous humour ions, it was not consistently correlated with the time of immersion. This means that there is no definite relationship between the rate of dilution of Mg, Ca, K, Na, and Cl ions in the vitreous humour and the immersion time. This might be due to individual variation in the rate of dilution of vitreous humour, which eventually restricts the methods. It appears, however, that magnesium in salt water cases and sodium and chloride in fresh water cases are related, albeit erratically, to the length of the immersion period.

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