15 Background Paper on Pathology—Collection, Processing and Storage of Pathological Material for Immediate Analysis and Later Study of Toxicological Effects and their Long-Term Implication

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15.1 INTRODUCTION

The pathological changes that take place in fatal and non-fatal cases of chemical exposures resulting from large-scale natural, accidental or man-made chemical disasters are not well known or described in forensic pathology. In recent years there has been a rapid growth of the chemical industry involving literally thousands of new chemicals. Correspondingly, a considerable body of information has been accumulated as to the safe handling of the several basic or raw materials, intermediates, reagents and finished products, varying vastly in quantity and reactivity. At the technological level, appropriate upscaling of safety measures at each state of chemical operation is taking place with the advent of automation and process control. Progressively more and more rigorous criteria and standards in processing, production, storage, transport and end-uses are being evolved over a period of time. Stricter standards have naturally been laid down for hazardous chemicals. Nevertheless, due to accidental causes or failures of man or machine, chemical accidents do occur caused by chemicals, intermediates or breakdown products. They are associated with either acute or chronic or long-term adverse effects in man, animals or even plant life. In some instances, chronic toxicity takes a long time to be manifested.

The characterization of the morbidity, the identification of the causative agent, and the understanding of the cause-and-effect relationship of the
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The study of toxic neuropathies, industrial dermatitides and organ-specific pathological damage is difficult, time consuming, multi-disciplinary and involves a variety of techniques and methodologies. It is difficult to find suitable experimental models or biomonitoring agents which could serve as indicators of alterations in the environment, viz. the air, water, soil and foodstuffs. The classical history of Minamata disease and acrylonitrile poisoning in industrial workers and chronic cyanide poisoning resulting in populations subsisting on cassava, are recent examples of industrial and food toxicology. New and unrecognized disease entities both in human beings and normal or experimental animals, supported by chemical and histopathological studies, has brought a better understanding of the underlying tissue damage, its mechanisms, and eventually the causative factor(s). This area of chronic chemical toxicity in man, in macro- and microfauna, or experimental studies, with emphasis on histopathology, is a subject which deserves special discussion, as in the case of acute chemically induced suicidal or homicidal poisons.

15.2 THE SCOPE OF PATHOLOGY IN CHEMICAL DISASTERS

Sudden, immediate, or large-scale accidents caused by bulk chemicals at different stages of their production, processing or accidental release into the surrounding environment, when accompanied by direct or indirect contact with living beings, including domestical animals and above all men, may cause unprecedented mortality and morbidity. The great Bhopal tragedy is a stark reminder of the need to take abundant precautionary measures and have a system for forecasting and forewarning of the population at risk and of the need to institute prompt and timely therapeutic measures. Perhaps the Bhopal gas leak is the single largest chemical disaster known to man. It has drawn worldwide attention on several counts, such as the population affected, the number of immediate deaths of men and animals such as buffaloes, goats, poultry, etc., continuing or progressive impairment of human health, and other long-term sequelae.

15.2.1 Certain Experiences from the Bhopal Disaster

The main chemical involved in the Bhopal disaster was no doubt methyl isocyanate (MIC). From the first week there were serious questions as to the nature of the chemical compounds involved. The possible role of pyrolytic breakdown products of MIC, such as HCN, CO, etc., of reactants like methyl amine, dimethyl urea, and of a series of polymerized compounds as later revealed by chemical analyses of the left-over residues in the fateful Tank No. 610 was questioned on the basis of forensic observations. The pathological
changes observed in the autopsies performed on the Bhopal gas victims and the dead cattle, if any, pointed towards a wider and more specific target organ damage than was expected from the available or subsequent toxicological information about MIC itself. Indeed these necropsy and histopathological observations led to interesting scientific information such as elevated excretion of urinary thiocyanate which was further enhanced under the influence of sodium thiosulphate on the one hand and demonstration of carbamylated haemoglobin and related changes in blood gases. The details of these studies in fatalities and survivors among men and cattle is bound to provide a better understanding of the disaster and an illustrative example of the ravages and sequelae wreaked by a single chemical and its derivatives. Apart from the basic chemicals, a whole range of unanticipated derivatives, some of which are likely to be more toxic or lethal, must be considered. The somewhat limited toxicity of the parent compound like MIC, however systematically studied, becomes somewhat academic and obscures the picture as subsequent events have revealed.

15.2.2 The Need for Cumulative Study of Small On-Site Accidents

Very often smaller accidents due to the spillage of chemicals such as oleum, ammonia, chlorine and other gases and chemicals occur sporadically and hence are virtually ignored. Chemical accidents are not necessarily confined to the factory or plant sites or during transportation in containers or vehicles or storage depots. They can occur as a consequence of accidental fires wherein modern sophisticated or polyurethane-based upholstery undergoes combustion to yield toxic fumes including HCN and CO, as in aircraft accidents or hotel fires.

Specific and consistent alteration in clinical and chemical pathology of small or isolated chemical accidents resulting in fatal or non-fatal consequences in men and animals in the vicinity of a potentially hazardous site of a chemical plant may provide vital clues towards understanding the chemical pathology of such agents. Such studies would also be helpful in the general treatment and assessment of specific antidotal therapeutic measures. It may be possible to generate basic information which would provide a better understanding of the metabolic derangements caused by chemical(s) and ultimately establish the 'biochemical lesions'. Thus pathological studies in a broader sense should encompass a wide gamut of information of both immediate and late consequences and thus help to produce a more comprehensive picture of tissue injury, pathological sequelae and the possible prevention of both. It is in that context that an attempt is made to outline the salient features of a programme of biological monitoring from the broad pathological point of view.
15.3 STUDIES AND COLLECTION OF DATA AND SAMPLES FROM SPORADIC, SEVERELY AND MODERATELY ILL PATIENTS AND CONTROLS

15.3.1 Chemical Information

Inventory of Chemicals

There should be an industry-wide inventory of all the chemicals used, the quantities stored, the process schedules, the temperature ranges over which the chemicals may undergo physical transformations from the solid to liquid to gaseous states, etc.

Data on Chemistry and Physical Chemistry

Essential and updated information should be made available about the interactions that occur and the end-product(s) produced during the normal operation schedule as well as alternate mechanism(s) that are likely to be encountered as a result of changes in operational conditions such as pressure, temperature, catalyst(s), etc. It should be made available preferably in a non-technical and easily understandable manner to health authorities and forensic pathologists and toxicologists in the community.

'On-site' Monitoring for Leakage of Dangerous Chemicals

The possible risks of leakage of specific gases or spillage of liquids like H₂S, SO₂, NO₂, nitriles, HCN, etc., should be periodically monitored with the help of on-the-spot test-kits. Although such information might not directly come under the purview of pathology, it will be useful in the further interpretation of pathological changes.

15.3.2 Record of On-Site Chemical-Induced Deaths and Sickness

Specific Register of Chemical-Induced Accidents

Every effort should be made to maintain comprehensive records of all minor and major, fatal and non-fatal, accidents, and also of chronic illnesses such as dermatitides, asthma, etc., among the workers in comparison with people of comparable economic and social habits such as those consuming alcohol and tobacco. Such information should be periodically reviewed and updated.

Updated Resume of On-site First-Aid Therapeutic Measures

Concurrent with the 'first-aid' measures that are provided in any emergency,
every effort should be made to observe, record and document any unusual features which tend towards specific organ involvement such as that of the eyes or of the cardiovascular system resulting in collapse, shock, etc., or involvement of the central nervous system characterized by seizures or other criteria.

Therapeutic Responses of ‘First Aid’

The therapeutic response to treatment such as symptomatic and supportive or specific and above-all antidotal measures should be recorded.

15.3.3 Clinical Investigations of Patients Exposed to Chemicals

Records of Clinical Examinations

The clinical investigations should include, in addition to a normal or standard clinical examination, a check-up for special signs and symptoms that are likely to be manifested in the case of the specific chemical industry. Gross appearance such as lividity, unusual cyanosis or reddish discoloration of the lips and oral mucosa may offer useful clues as noted in some of the initial findings in Bhopal.

15.3.4 Clinico-Pathological Information

Routine Laboratory Investigations

The clinico-pathological studies should cover the gamut of examination of the blood and urine, including presence or absence of haemodilution or haemocoagulation, leucocytosis or leucopenia and abnormalities in urine such as gross alteration in pH, proteinuria, presence of biotransformed conjugates like glucuronide, thiocyanate, etc.

Specialized Investigations

Contents of vomitus, stomach washes and lavages (where indicated) of the initial victims should be carefully collected and preserved and forwarded for immedical chemical and cytological studies. The type of specimens to be collected for chemical analysis depends on the chemical which caused the outbreak. For certain metals, preserve hair, nails, teeth (e.g. for lead or mercury content) in children, fatty tissue for chlorinated hydrocarbon compounds. For unknown chemicals, stomach content, liver bile, kidney fatty tissue, brain, urine, blood, aspirated from heart if possible, hair and bone. Containers should be clean; each specimen should be in a separate container. If metals (particularly nickel carbonyl) are suspected, plastic knives need to be used for cutting. Nalgene containers are best for chlorinated hydrocarbon
compounds. If glass containers are used, protect tissues from the plastic in the lids. Large specimens should be collected, if possible at least 100 grams/organ. They should be cut into pieces before freezing for easier removal from the container. Every effort should be made to preserve samples of urine, whole blood, plasma or serum and erythrocytes, washed in saline, not only for immediate testing but 'cryopreserved' for subsequent analyses. If the patient was hospitalized, any blood specimens and urine collected while the patient was still alive which may still be in refrigerators at the nursing station or the hospital laboratory should be collected and frozen. If dealing with a new disease of truly unknown cause, all materials should be treated as potentially infectious. Proper precautions should be taken and appropriate cultures for infectious agents should also be taken.

Schedule of Investigations

A schedule of standard investigations and special tests to be carried out on the biological fluids should be worked out based on available information and previous experience.

Preservation of Samples for Future Studies

Since it would not be known at the initial stages as to the type of investigation(s) that would be carried out later on, it is extremely important to preserve adequate samples of the above biological fluids from the dead as well as living patients. If necessary duplicate samples of the biological fluids of living patients may be preserved with a view to carry out tests that may not be foreseen at the time of the disaster. In any case all samples of biological fluids thus obtained should be preserved at as low a temperature as possible, generally $-25^\circ$C, if not at $-90^\circ$C.

15.3.5 Clinical Illustrations

Colour photographs of the patients depicting the clinical appearances could be an invaluable record of some of the salient findings.

15.3.6 Continuing Studies of Individuals at Risk

In addition to the patients who are critically ill, all suspected patients and persons at risk and those who are in the vicinity of the chemical disaster should be enumerated and samples of urine, blood, etc., should be collected and preserved just as in the case of the clinically affected individuals. In fact, it would be desirable to collect samples for control populations in nearby places. Such baseline information would be hard to obtain later. However, this aspect
can only be attended to after the immediate care of the patients brought to the 'medical emergency'.

15.4 POST-MORTEM OR AUTOPSY STUDIES

Nowhere is the dictum that 'the dead teach the living' of greater relevance than in the case of medical disasters. This is a major lesson from the Bhopal disaster. Due to the untiring efforts of a dedicated team of forensic pathologists, a large amount of information was collected, documented from the third day after the gas leak onwards. Based on the experiences gained, the following suggestions are made.

15.4.1 Identifying Illustrations

With a view to establish identity of the victims, clinical photographs with suitable identifying number plates should be taken. This helps in the identification of a very large number of dead persons, a formidable task in the midst of a disaster. It is of great value in the assessment of the casualties, defining areas of greater vulnerability and claims for compensation.

15.4.2 Physical Examination of the Body

The gross pathological findings such as rigor mortis, lividity post-mortem hypostasis and evidence of cyanosis of the finger nails, lips and the mucosa, and the character, nature, odour of the secretions accumulating in the nostrils or nares should be recorded. Presence of conjunctival haemorrhage, etc., should be not only documented and recorded but also photographed, in colour wherever possible.

15.4.3 Chest X-ray

Before the post-mortem is done, every single body should be subjected to a chest X-ray, which would provide baseline interpretation of the study of the lungs, nearer the time of death.

15.4.4 The Autopsy

The autopsy must be meticulously performed according to standard forensic methods with the following special considerations.

Air Sampling

Even before opening of the thoracic cage, it might be worth while to aspirate the 'dead space air' from the tracheobronchial tree, with the help of suitable
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diagnostic aspiration pump sets for testing even the small quantities of chemical irritants like \( \text{H}_2\text{S}, \text{SO}_2, \text{HCN} \), etc.

The Brain

It is necessary to decide as to the manner in which the brain should be preserved/fixed. It is likely to be affected by the chemical poisons either primarily or secondarily as a consequence of anoxia due to cardio-pulmonary causes. It may be desirable to cut the brain sagitally into two halves and preserve one in a suitable polythene bag, duly labelled, and then preserve at a very low temperature (\(-25^\circ\text{C}\) and below). Such a procedure would be particularly necessary for the detection of cyanide, if suspected. The other half of the brain should be suspended in abundant quantities of 10% formal saline followed by frequent changes of the fixative to enable systematic studies to be carried out in a routine manner, based either on paraffin or celloidin sections. It may be important to add that while handling the brain, tiny representative samples of tissue from the cerebral and cerebellar cortex and the white matter should be collected immediately for electron microscopic studies. It must be ensured that the specimens taken for this purpose do not exceed 1 mm\(^3\) and are transferred at the earliest opportunity to the appropriate glutaraldehyde fixative in suitable vials which are appropriately labelled.

Preservation of Tissues for Chemical Analyses

Adequate samples of the major viscera like the lungs, brain, liver, kidneys, spleen, etc., should be deep frozen without any addition of a fixative for subsequent chemical analyses by refined and sensitive techniques such as high pressure liquid chromatography (HPLC) and gas chromatography (GC).

Collection of Urine at the Time of Autopsy

Special reference is made to the collection of the contents of the urinary bladder at the time of the autopsy, since it would be a useful reflection of the nature of the metabolic waste products immediately preceding death. Such urine samples should be subjected to immediate analysis for pH, proteinuria, presence of casts, etc., and the rest preserved at low temperature for subsequent examination of specific metabolites, drug intermediates or specific chemicals which are either conjugated, detoxified or excreted as such.

15.5 HISTOPATHOLOGY

15.5.1 Routine and Special Staining Procedures

The material for electron microscopy and for light microscopy should be
processed strictly according to the conventional or classical methods of tissue processing and staining by routine Haematoxylin and Eosin as well as special staining procedures. Representative sections should be stained by a battery of histological techniques for the study of cellular and extracellular components, including collagen, reticulin and elastic fibres in the case of the lungs and Nissl an other special procedures in the case of the nervous tissues.

15.5.2 Special Tissues

In view of the greater likelihood of poisons affecting either the central nervous system or more particularly the peripheral nervous system, and the neuromuscular apparatus, care should be taken to dissect the selected tissues suitable for further study.

15.6 BIOCHEMICAL ANALYSIS OF TISSUE SAMPLES

It is absolutely necessary to collect, as in the case of the living patients, representative samples of biological fluids such as blood, urine, etc., or aliquots thereof for immediate investigation and subsequent verification and/or special tests. Deep frozen blocks of tissues from the lung, liver, brain, etc., should be made available for chemical, biochemical, enzymatic and pharmacokinetic studies and special investigations such as HPLC and GC for detection of specific compounds. Specialized investigations such as the presence of cyanide and acrylonitriles or their intermediates and decomposition products or materials representing biotransformation can be investigated only when the fresh frozen unfixed material of the initial victims at the time of the disaster are carefully preserved.

15.7 IMMUNOLOGICAL STUDIES

Representative samples of blood and sera should be carefully preserved at low temperatures for subsequent immunological studies, particularly regarding the distribution of immunoglobulins and other antigens. This is particularly important with regard to screening for hypersensitivity reactions caused by the chemicals following inhalation or more likely dermal contact. It is hoped that with ever-improving immunological techniques and their sensitivity, it would be possible to arrive at sensitive parameters in immunoglobulin profiles.

15.8 CONTINUED POST-MORTEM STUDIES

While the immediate post-mortem studies would be of very great value, subsequent deaths in the affected or exposed population would be of similar, if not greater, importance. This applies to the sub-acute or chronic lesions among
the late victims and the survivors. By then some prima facie knowledge or information would have already been gained based on the initial studies. Hence, the sampling, preservation and testing of the material can become more specific and localized. By way of illustration, reference is made to alterations in the neuromuscular junction. In the case of inhalation toxicity, consequences of changes and the deterioration in the lungs, etc., would assume greater importance. Information such as sequelae of selective neuronal damage in the brain and accompanying glial reaction or persistent cerebral oedema would be of inestimable value. Consolidation, resolution or fibrosis of pulmonary lesions must be specifically looked for. Similarly the sequential changes in the liver may throw light on the metabolic changes brought about. Hypersensitivity as shown by cutaneous manifestations needs further studies.

15.9 LONG-TERM FOLLOW-UP STUDIES

Long-term follow-up among survivors is yet another major study of the pathological processes following a chemical disaster. Suitable tissue samples of suspected tissues such as skin biopsies, liver biopsies and above all lung biopsies would be of great value in the assessment of the late sequelae of the poison(s).

15.10 TERATOGENIC AND MUTAGENIC STUDIES

There is certainly a possibility of teratogenic manifestations apart from the foetal losses. A careful watch of expectant mothers and the study of all the aborted foetuses including regular monitoring of intrauterine growth followed by neonatal and infant studies is necessary. Although not strictly part of pathological investigations, they should not be lost sight of. In fact, it would be eminently desirable to collect cord blood samples of all the derivatives that take place in affected areas and either immediately test or preserve the specimens for more specific investigations to supplement information obtained in adults.

15.11 ANIMAL TISSUES

Last but not least, in addition to human victims, it is equally important to collect and collate pathological studies from different species of animals such as cows, goats, dogs and poultry in the vicinity of an accident. The blood and tissue samples should be subjected to the same critical evaluation.

15.12 CONCLUSIONS

In any study of the adverse effects of hazardous chemicals and of injury due to chemical accidents, there is a need for a very careful, well-organized, planned
pathological study of all the available tissue materials and biological fluids of
the actually ill, dying and early and late victims as well as survivors caused by a
wide variety of chemicals with the help of light and electron microscopic,
immunopathological and above all chemical and special techniques of organic
chemical analyses.

General references are included in the Appendix in Part A of this volume.