

ANALYSIS OF BIOLOGICAL MATERIAL IN INDUSTRIAL TOXICOLOGY

ANA SINGERMAN

Toxicology Research Laboratory, Buenos Aires University, Argentina

INTRODUCTION

Occupational hazards have existed since the beginning of technology and they have always been considered part of the job. At the present time a different idea and a modern concept about the importance of each individual's health among the working class, has brought about a transcendental change in labour legislation. However, the workmen's compensation laws are not effective enough from either the sanitary or the toxicological point of view. It is necessary to think of detecting any possibility of physiological changes or metabolic disturbances leading to an industrial poisoning, at an early stage, in order to apply corrective technical measures in the working atmosphere thus avoiding the disease long before the symptoms appear. And there is only one way to achieve this purpose: taking advantage of the numerous new laboratory tests which are constantly being developed.

Sometimes, months or years of exposure may elapse before toxic manifestations appear. In the meantime the offending agent will be acting upon the sensitive receptors, causing a series of small insults—usually not perceptible—to a group of cells. None of us ignore the extraordinary and ever increasing significance attributed to the enzymes. The understanding of these protein molecules will allow us to know the metabolic activities of living organisms and throw some light into the biological mechanisms by means of which health or disease will show as their dominant feature.

When an injurious chemical agent is present in the atmosphere of a working place, the workers are exposed to an occupational disease. An equilibrium is generally established between absorption and excretion, the result of which will be the presence of the chemical and or its metabolites in tissues and body fluids. The determination of the injurious agent in air samples and body fluids may give an indication of the effectiveness of the preventive measures, especially in cases where a good correlation exists between air, blood and urine levels. When complete information is needed in order to give a "biochemical diagnosis", invaluable for the early detection of an occupational disease during the asymptomatic stage, it is necessary to include more apt tests to reveal the slight changes occurring in enzymatic activity due to the presence of small amounts of that injurious agent in the cells.

The use of various biochemical tests for the study of the mechanism of action of poisons is taking an ever greater importance in modern toxicology. Of course, the use of such methods requires highly qualified interpretation.

The limits of harmless fluctuation, lying within the range of physiological variation, have been set for a number of enzymatic systems. This information may be used to evidence any change in their activity as a consequence

of a cell injury. Here, it is necessary to point out the fact that repeated determinations on the same individual range around a value which is characteristic of that individual, and this fact is not generally taken into consideration by the most part of statistical investigations of this nature.

Enzymes are not homogeneously distributed throughout a cell but are concentrated in certain compartments or subcellular structures. Otherwise, the numerous cell reactions could not be carried out simultaneously in an organized manner. When a toxic agent enters the cell a disturbance may occur in the enzymatic regulation of the vital cellular process. Even though detailed information is still lacking, many of the most important aspects of Enzymology have been already studied. Information obtained on isolated enzymes may be extended to the same enzymes as integral units of the cellular complex. The studies carried out on enzyme inhibitors have widely contributed to the knowledge of many enzyme mechanisms, as well as to the elucidation of the various aspects of cellular metabolism.

METHODOLOGY

Advances in Biology and Biochemistry in the last years have required the development of extremely sensitive analytical procedures in order to investigate and follow the way of minute amounts of chemical agents within tissues and cells, as well as to penetrate into the intracellular mechanisms of activity.

Toxicology, as a highly specialized branch of Science closely related to Biochemistry, has taken advantage of the most refined analytical procedures, trying to apply them for its specific purpose. Modern techniques, particularly through their extension into the micro and ultramicro range, have greatly contributed to the biochemical diagnosis of exposure.

It is very important to keep in mind that laboratory data are only the result of a number of determinations carried out on specimens obtained at a certain moment, and not to forget that workmen's exposure may vary either hour to hour or day to day according to the various tasks they have been assigned to.

Toxicological phenomena cannot be satisfactorily explained on the basis of only a few mathematical data. A good knowledge of Biology and Biochemistry is required to avoid misinterpretation.

It may be of interest to examine briefly the modern methods being applied in specialized laboratories for the prevention, control, and diagnosis in Industrial Toxicology. Among the many instrumental methods daily used in our field, we will give a general idea on only a few of them having been either introduced or perfected in the last decade.

POLAROGRAPHY

The principle of polarography consists in the use of a mercury drop electrode for electrolytic analysis, particularly with variable potentials. Quantitative and qualitative analyses of substances are possible if the substance in question is capable of undergoing cathodic reduction or anodic oxidation. Polarographic curves give an indication of the identities of the substances present in the material to be analyzed as well as of their concentrations.

Polarography is a method giving a relatively high sensitivity, having been used for the determination of various metals such as lead, cadmium, cobalt, manganese, arsenic, antimony, etc., in blood and in urine. Carbon disulphide, benzene, toluene, dinitrophenol, and other organic compounds have also been determined in biological materials by means of polarography. New different instruments and techniques have been developed in recent years—including polarography of radioactive isotopes—claiming for increased sensitivity and simplicity as compared with classical polarography.

The polarographic method is suitable for a quick series of determinations. Since it is possible to determine concentrations as low as 10^{-6} M for many substances, the sensitivity of the method is recommended for the determination of traces of elements in Industrial Toxicology.

GAS CHROMATOGRAPHY

Few novel techniques have ever had as spectacular a growth as gas chromatography has had in the last few years. Gas chromatography is a method by which a mixture of substances in very small quantities can be rapidly separated and examined by using a gas as the moving phase. The sample size required for the gas chromatograph is micro, because of the extremely high sensitivity of detectors used. Analyses by gas chromatography are very fast and accurate, and can be made quite specific.

In applying gas chromatography to analytical separations, there are three basic techniques: (i) adsorption-elution chromatography; (ii) gas-liquid partition chromatography; (iii) adsorption-displacement chromatography. A great deal of work has been done on different types of columns, liquid phases, solid supports, adsorbents, detectors, etc., in order to obtain higher resolutions, and many instrumental improvements have been achieved. Dominguez *et al.* have developed a sensitive and specific method for the determination of carbon monoxide in blood by gas chromatography. Many volatiles such as alcohols, aldehydes, ketones, carbon disulphide, etc., have been isolated and quantitatively determined by gas chromatography.

Gas chromatography has become so accepted in pesticide analysis that it is considered today as the method of choice. Radomski and Fišerova-Bergerova have applied gas-liquid chromatography to the petroleum ether extracts of tissues for the determination of chlorinated pesticides, reporting a sensitivity ranging from 0.001 to 0.06 ppm. Kadis and Jonasson have also applied gas-liquid chromatography to the determination of chlorinated pesticides in blood, by previously chromatographing the sample in a Florisil column with 30 per cent methylene chloride in petroleum ether, evaporating the eluate and injecting the hexane solution of the residue into the instrument. Jane, Fontan and Kirk, after extracting the blood sample with an acetone-ether mixture, evaporating, taking up in hexane, and injecting into a chromatographer, have succeeded in determining 23 different pesticides including chlorinated and organophosphorous compounds.

Gas chromatography is very often supplemented by means of infrared spectrometry in the identification of many industrial poisons.

NEUTRON ACTIVATION ANALYSIS

Activation analysis is a technique which permits the determination of many elements at levels well below the limits of conventional analytical methods. Activation analysis can be used for the estimation of any element. For most elements, activation by thermal neutrons is the method of choice. When this approach does not give adequate sensitivity, fast neutrons or charged particles like protons or deuterons may be used. Neutron activation analysis is the most sensitive method known to date for the detection of low concentration of elements. The sensitivity is sometimes 100 to 1000 times that of any other method.

This method of elemental analysis is based upon the production of radioisotopes in the sample to be analyzed, by nuclear reactions induced by neutron bombardment, followed by the identification and quantitative estimation of the different radioisotopes formed. The elements H, C, N and O which together constitute the 96 per cent of all biological material, are not activated by thermal neutrons. This fact makes activation analysis extremely useful to determine trace elements in biological material. The activation analysts have devoted to the investigation of new methods of irradiation, counting, and data processing, trying to improve the known techniques, in an effort to increase accuracy and precision.

Manganese in urine has been determined by neutron activation analysis by Moav; arsenic in biological samples has been determined by Krishnan and Erickson as well as by many others, applying neutron activation analysis. Kellershohn, Comar and Le Poec have measured mercury in blood by activation analysis. Livingston and Smith have estimated vanadium in biological material by neutron activation analysis.

X-RAY METHODS

When a rapidly moving electron impinges on an atom it may knock an electron completely out of one of the inner orbits of that atom. When electrons from the outer orbits fall to the vacant inner orbit, the difference in energy is emitted as x-rays. X-rays are a form of radiant energy with very short wavelength, which can be employed in analytical work. If an unknown material is used as the target in an x-ray tube, the characteristic lines of the elements in the unknown will be produced, and if an internal standard is used the relative intensities of lines may be measured. These are the bases of x-ray spectrophotometry, and of x-ray spectrography.

When working with biological material the element sought is either concentrated by ion-exchange chromatography or extracted with a chelating agent such as dithizone. Sometimes the element is also concentrated by electroplating prior to x-ray spectrometry.

Amounts ranging from 1 μg to 100 μg of heavy metals may be analyzed successfully, and a sensitivity of less than one microgram may be obtained for some of them. Vanadium, chromium, nickel, mercury, etc. have been determined by x-ray spectrography. Limits of detectability range from 0.001 to 10 μg for elements that have been previously isolated from the biological material.

Swedish authors have recently developed a multichannel x-ray spectrophotometer for analyzing *in vivo*. Their instrumentation is intended primarily for medical research, but the principles are applicable to other analytical problems.

ATOMIC ABSORPTION SPECTROMETRY

Atomic absorption spectrometry is a highly sensitive, specific, and simple method of analysis. It was not until 1955 that Walsh recognized the advantages of the absorption method and devised a simple, versatile apparatus applicable to the routine analysis of solutions of a wide range of elements.

Walsh showed that the phenomenon of atomic absorption could serve as a spectrochemical basis for the quantitative determination of traces of metals. He demonstrated that the measurement of metals by atomic absorption spectrometry is more sensitive than measurements by flame emission spectrometry and less subject to interference from other elements.

The production of atoms from a molecule of a chemical compound requires the absorption of energy, which is usually supplied in the form of heat. When vaporized by heating in a flame, a compound is partially or wholly broken up into its elements in the gaseous form, and some of the atoms are further excited to a state from which they can emit radiation on returning to the unexcited, or ground state. On the other hand, atoms in the ground state can absorb radiation only at a limited number of wavelengths, corresponding to their resonance lines. Atomic absorption is similar to emission flame photometry, except that instead of measuring energy emitted by atoms it measures energy absorbed by atoms.

The atomic absorption spectrometry is used for the determination of trace quantities of metals in biological fluids. Some of them can be measured directly in urine samples. Others require a previous extraction into an organic solvent after chelating the metal, by means of which procedure a concentration factor of about 100 may be obtained.

Atomic absorption spectrometry has been successfully applied by Berman to the determination of lead in blood and urine, and by Pierce and Cholak to the determination of lead, chromium and molybdenum in urine and blood. Sunderman has measured nickel in biologic materials by atomic absorption spectrometry. Kinser has determined concentrations as low as 1.5 μg of bismuth and 2.0 μg of tellurium in one gram of animal tissue by atomic absorption spectrometry.

ENZYMATIC ANALYSIS

Nearly all biological reactions are enzyme-catalyzed and subject to a variety of cellular controls. The measurement of enzymatic activity is an extremely sensitive technique to evidence abnormal metabolism and slight cell injuries. This exquisite sensitivity derives from the fact that enzymes catalyze the metabolic reactions and it is precisely the catalytic rate which is being measured. A minute amount of enzyme brings about the chemical alteration of a large amount of substrate per unit time.

Enzymatic analysis generally means analysis performed with the aid of enzymes, but it also includes the assay of enzyme activity in organs and biologic fluids. An enzyme is capable of catalyzing a reaction of a substrate,

even though other isomers of the substrate may be present. The value of enzymes in analysis lies in their ability to react specifically with individual components of a mixture.

Enzyme-catalyzed reactions have been used for the determination of substrates, activators, inhibitors, and also of enzymes themselves.

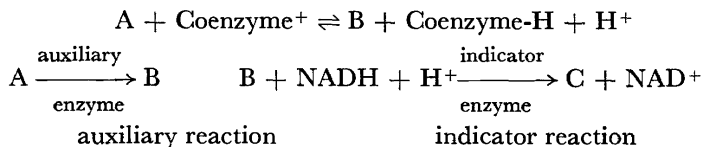
The advent of new techniques and the automation of enzymatic reactions have increased the speed, ease and reproducibility of enzymatic analysis.

When the concentration of a substance is to be determined by means of enzymatic analysis, two different groups of methods may be applied: (a) by the measurement of total change, after completion of the reaction catalyzed by the enzyme; (b) by the kinetic method, calculating the rate of the enzyme reaction, which depends on the concentration of the substrate, cofactor, activator, and/or inhibitor.

Methods measuring total changes

When a substance A which is enzymatically converted to a substance B is to be determined, it may occur that A has a characteristic absorption spectrum. In this case, the absorption will decrease by an amount corresponding to the amount of A. When A does not absorb, the enzymatic conversion of substrate A can be followed by the transfer of hydrogen of A to the pyridine ring of a cofactor (NAD) leading to the formation of a peak in the absorption curve.

Sometimes the substance A to be determined is converted to B with an auxiliary enzyme and subsequently coupled with an indicator reaction employing an indicator enzyme, B being the substrate for a NAD-linked dehydrogenase. NADH is oxidized and the decrease in absorption is determined.



Methods measuring the kinetics of the reaction

The rate of an enzyme reaction depends, within certain limits, on the substrate concentration, and is controlled in the living cell by different regulators. An activator is a small molecule, generally an inorganic ion, required for an enzyme to be an active catalyst. Unlike the coenzyme, the activator is not itself an explicit participant in the reaction. The activity of the enzyme increases until enough activator is used to activate the enzyme fully. With constant amounts of enzyme the rate of the reaction is dependent on the activator concentration.

An inhibitor is a substance producing a decrease in the rate of an enzyme reaction, either by reacting with the enzyme to form an enzyme-inhibitor complex or with the enzyme-substrate intermediate to form a complex. The initial rate will decrease with increasing inhibitor concentrations.

Numerous enzymes require the presence of a small non-protein prosthetic group linked to the protein and termed cofactor or coenzyme, in order to perform efficiently the catalytic process. The coenzyme, as well as

the substrate, may be altered in the course of the reaction, although the original structure is usually regenerated. In coenzyme-dependent reactions the binding of coenzyme to enzyme precedes substrate-enzyme interaction.

Because it is more reliable, the total change method is generally adopted. However, the method can only be used for substrate analysis and not for enzyme, activator and inhibitor, which are catalytic in nature and affect only the rate but not the equilibrium. The rate method is faster because the rate can be measured initially without having to wait for the reaction to go to completion. In order to obtain the maximum sensitivity it is necessary to carefully control the conditions affecting the rate, i.e. pH, temperature, ionic strength, etc.

A relatively new method for assay of enzymes is the measurement of the amount of fluorescence liberated from a non-fluorescent substrate under standard conditions. Since fluorogenic substrates are much more sensitive than chromogenic ones, a significant increase in the sensitivity of the assay will be obtained. Different non-fluorescent esters of fluorogenic compounds can be hydrolysed by enzymes to give fluorescent substances.

Another possibility is to measure the change in fluorescence produced by the reduction or oxidation of pyridine nucleotides in the coupled reaction, when dehydrogenases requiring either NAD or NADP as hydrogen acceptors, are to be determined. Since NADH and NADPH have a high native fluorescence, many fluorometric procedures have been developed using the NAD-NADH and NADP-NADPH systems.

A similar method is based on the conversion of resazurin (non-fluorescent) to resorufin (highly fluorescent) in conjunction with the NAD-NADH system. An increase in sensitivity is obtained due to the intense fluorescence of resorufin (as little as 10^{-9} M can be detected). Automated systems are frequently applied for the determination of enzyme activity.

According to Schwartz and Bodansky, three stages of automation may be considered to exist. The first stage is the one in which the measurement of enzyme activity is instrumentally recorded, being the rest of the technique manually prepared. The second stage involves instrumental recording as well as automated preparation of the reaction mixtures. The third stage of automation would incorporate feedback devices to control and correct the action of the instrumental components and transform the enzyme activity into numerical values.

The automated assay of the activity of many enzymes may be accomplished with the auto analyzer, recording the absorbancy in a colorimeter, the u.v. absorption in a spectrophotometer or the fluorescence in a fluorometer.

Murray and Harmon have described at the 18th National Meeting of the American Association of Clinical Chemists held in 1966, a completely automated system for the determination of enzyme activity by initial reaction rates. The instrument automates the entire procedure from sampling to calculation of final results.

Isoenzymes

In the course of the last decade attention has been directed to the elucidation of the heterogeneity of enzymes. Since the remarkable observation by Markert and Møller in 1959, about the multiple forms of enzymes, different

investigators have been intensively working on the subject, and increasing evidence has been established about the multiple molecular forms of enzymes in tissues and cells.

The different molecular forms of enzymes, constituted by proteins with similar catalytic properties, acting on the same substrate, but with different physico-chemical and immunological behaviour, are known as isoenzymes. The isoenzymes are located in different intracellular compartments, and they may be either cytoplasmic or mitochondrial.

If the part of the mitochondrial isoenzymes in serum is determined with respect to the total activity of the enzyme, it is possible to detect the degree of disturbed permeability of the mitochondria, revealing thus the nature and severity of the damage of the cells involved.

The serum from normal adult individuals contains a definite number of isoenzymes in a characteristic pattern. When a tissue is damaged it contributes the isoenzymes contained therein, resulting in a shift of the serum isoenzyme pattern from the normal towards an abnormal pattern showing an increase in the isoenzymes present in the injured tissue.

The isoenzyme pattern is controlled not only by genetic determinants but also by physiological or metabolic requirements of individual organs and tissues.

The study of isoenzymes by chromatographic and electrophoretic methods has allowed a clearer understanding of various aspects of enzymatic phenomena. Advances in this field will probably give the clue for many toxicological problems the elucidation of which constitutes the aim of most of us.

Allen has shown that certain isoenzymes of lactate dehydrogenase are distributed in characteristic locations within the cell and that they differ somewhat in enzymatic specificity. Likewise the two main isoenzymes of malate dehydrogenase are localized in the mitochondria and in the cytoplasm, respectively, according to Thorne. These findings suggest that the distribution of each isoenzyme to characteristic positions within the cell would enable them to perform distinctive metabolic roles even though their enzymatic properties were very similar.

There are strong reasons to support the idea that the careful analysis of the enzymatic systems involved as well as of their isoenzymes patterns may shed some light on the mechanism of action of a number of poisons, leading thus to the diagnosis of a pretoxic condition, and this seems to be an extremely promising field in Industrial Toxicology.

HYPERSUSCEPTIBILITY DETECTION

The studies being carried out on the individual hypersusceptibility to industrial chemicals and drugs are of the greatest importance in our field. Inherited variations in man leading to variations in the response to drugs have been recognized for many years, but it has been only during the last ten to twelve years that some of these differences have been investigated by pharmacologists, geneticists and biochemists.

According to La Du most of the inherited conditions which are associated with altered drug metabolism or drug response may be grouped as follows: (i) hereditary disorders associated with a deficiency of a specific enzymatic

system (inborn errors of metabolism); (ii) hereditary disorders associated with a deficiency or an alteration in the structure of a specific functional protein (molecular diseases); (iii) hereditary defects in regulatory mechanisms which control the rate of protein synthesis; (iv) hereditary or congenital conditions associated with gross or microscopic structural defects.

Inborn errors of metabolism are generally explained by hereditary enzyme deficiencies leading to an abnormal biochemical behaviour.

It is well known that methemoglobin is reduced by NADH_2 to haemoglobin at a slow rate. In normal cells the reduction is catalyzed by the methemoglobin-reductase. An erythrocytic deficiency in the enzyme activity may raise the level of methemoglobin from less than 2 per cent to values ranging between 5 and 50 per cent, and a marked methemoglobinemia is developed if nitrites or sulfonamides are given.

Glucose-6-phosphate dehydrogenase is the enzyme that oxidizes the glucose-6-phosphate to 6-phosphogluconate. The removed hydrogen is accepted by NAD and then by oxidized glutathione through glutathione reductase. Thus, a genetic deficiency of glucose-6-phosphate dehydrogenase activity in the red blood cell leads to a decrease in NADH_2 and GSH which are critical cofactors in maintaining the reduced cellular environment.

Normally, the cellular integrity is preserved, but under the stress of added demand for hydrogen by oxidized drug and chemical metabolites, haemolysis of the red blood cell may occur. Deficient subjects develop hemolytic anaemia upon administration of sulfonamides, *p*-aminosalicylic acid, phenacetin, etc.

The hemolysis is thought to arise because of an insufficient supply of NADH_2 required by the erythrocytic glutathione reductase, thus producing a constant deficiency of reduced glutathione, which seems to be necessary for the reduction of methemoglobin to haemoglobin.

Ramot and collaborators have shown that glucose-6-phosphate dehydrogenase requires an activator, and that defective cells lack this activator, appearing consequently as deficient in glucose-6-phosphate dehydrogenase activity.

It has been shown that the gene for glucose-6-phosphate dehydrogenase deficiency is probably located on the x-chromosome and is thus detected more often in males than in females, and it has a high frequency in some racial groups, but a low frequency in others. Affected males suffer from a more marked enzyme deficiency and haemolysis than do affected females. The enzyme deficiency appears therefore to be inherited through a partially dominant gene, which is sex-linked. In recent years at least ten sub-types of glucose-6-phosphate dehydrogenase deficiencies have been recognized. Kalow has reviewed some of the complexities of the problem.

Some deficiencies in glucuronide conjugation have also been described as inherited metabolic disorders characterized by hyperbilirubinemia and jaundice. A number of drugs which normally undergo glucuronide conjugation are conjugated less effectively by these patients.

Deficiencies or alteration in the structure of haemoglobins, plasma proteins, proteins involved in transport of metabolites, etc., are known as molecular diseases. Some haemoglobin variants cause an increase in the basal level of methemoglobin in the erythrocytes. Individuals with

haemoglobin H, for instance, which is easily oxidized to methemoglobin, develop an acute anaemia if given sulfonamides or nitrites, because of the accumulation of methemoglobin, followed by haemolysis of the red cells. It has been demonstrated that individuals with some molecular diseases may show an unusual susceptibility to toxic agents in the environment.

Concerning the inherited defects in regulation of protein synthesis, a good example is the hepatic type of porphyria. Barbiturates are able to induce an acute attack of porphyria in individuals with this inherited susceptibility.

As has been shown by Granick, the rate-limiting enzyme step of porphyrin synthesis in the liver is delta-aminolevulinic acid synthetase, which can be increased in tissue culture by barbiturates. Granick and Levere have recently suggested that the hereditary defect in patients with porphyria may be a faulty repressor system for the synthetase.

Inherited variations in gross anatomical or microscopic structure may be responsible for variations in drug response. It has been reported that in mongolism there is an unusual sensitivity to atropine and hydroxy-amphetamine. A greater pupillary dilatation to local atropine and hydroxyamphetamine has been shown, but it is not known whether the reaction is limited to the eye or represents a generalized sensitivity to these agents.

As has been explained above, certain individuals are highly susceptible to different chemicals. A number of workers hypersusceptible to the injurious effects of toxic agents may be found in different industrial plants. It is, therefore, very important to detect that hypersusceptibility and to relate it to the working environment.

A few laboratory tests have been applied for the detection of hypersusceptibility associated with defective glucose-6-phosphate dehydrogenase, namely: (i) the determination of glucose-6-phosphate dehydrogenase activity; (ii) the methemoglobin reduction test; (iii) the glutathione stability test.

The measurement of glucose-6-phosphate dehydrogenase activity by determination of the rate of reduction of NADP to NADPH₂ by suspensions of red cells gives a direct information, telling us whether the enzyme activity is normal, defective or elevated.

The methemoglobin reduction test of Brewer, Tarlov and Alving is recommended for routine use because of its simplicity. It is based on the inability of sensitive cells to reduce methemoglobin in the presence of glucose and methylene blue under aerobic conditions. The rate of reduction is therefore slower than normal. The test involves the oxidation of haemoglobin to methemoglobin by sodium nitrite and its subsequent enzymatic reconversion to haemoglobin in the presence of methylene blue. This redox dye affects the pentose phosphate pathway and activates NADPH₂-methemoglobin reductase in normal, but not in glucose-6-phosphate dehydrogenase deficient erythrocytes.

The glutathione stability test as developed by Beutler is based on the disappearance of reduced glutathione from erythrocytes during aerobic incubation with glucose and acetylphenylhydrazine. The result is expressed as the amount of reduced glutathione remaining at the end of the incubation period. When normal cells are incubated with the oxidative reagent near

normal levels of reduced glutathione can be restored. When a deficiency of glucose-6-phosphate dehydrogenase is present, the red cells have a poor capacity to restore reduced glutathione levels to that of untreated cells. Values below 40 mg/100 ml erythrocytes are considered abnormal.

Another problem, having a certain incidence on agricultural workers, is the one concerned with the familial reduction in plasma cholinesterase level as described by Lehmann and Ryan and by Kalow, and the familial reduction in red-cell cholinesterase as described by Johns. The red cells, muscles and nervous system contain acetylcholinesterase whereas the plasma contains a non-specific cholinesterase synthesized in the liver.

According to Johns, although the familial reduction in red-cell cholinesterase activity may resemble the plasma cholinesterase deficiency, there is no reason to suppose biologic, biochemical or genetic similarities between both problems.

In the plasma cholinesterase deficiency an abnormal cholinesterase has been separated from the normal esterase chromatographically and electrophoretically. In the reduction of red-cell cholinesterase activity it has not yet been possible to distinguish this abnormal esterase from the normal enzyme. Both deficiencies seem to be genetically controlled.

Although there is a low incidence of these familial traits in the population, individuals with such deficiencies may be susceptible to cholinesterase-inhibiting insecticides.

Measurements of blood cholinesterase activity (plasma and erythrocytes levels) are essential for the control of exposure to organophosphorous and carbamate insecticides.

The ability to detect a fall in blood cholinesterase due to exposure to an inhibitor, will depend on the natural fluctuation of the enzyme activity. Repeated determinations on the same individual show values the average of which is considered to be characteristic of that individual. A fall in red cell or plasma cholinesterase activity will be indicative of exposure. In those cases with a typical low cholinesterase activity, such a fall may lead to a hyper-response.

SUMMARY AND CONCLUSIONS

Toxicology is a branch of Science dealing with disturbances in vital biochemical systems and in normal metabolic pathways. In the field of Industrial Toxicology it has been stated by a number of authors that our attention and interest are being reoriented from the environment to the worker.

The analysis of the working atmosphere may provide a means of control for the average worker, but a better understanding of each individual's problem is obtained when a careful study of his biochemical systems is performed. The analysis of the multiple inorganic and organic poisons and their metabolites in the biologic media of the worker is providing a good deal of information, but if the aim is to detect an abnormal absorption of a toxic substance long before the clinic symptoms appear, the variations in the activity of different enzymatic systems involved as well as of their isoenzymes patterns have to be obtained.

On the other hand, the identification of individuals with a high risk for development of certain diseases as a consequence of hereditary enzyme deficiencies, is becoming a very important chapter of Industrial Toxicology. Different tests are being developed to detect susceptible individuals within the working population, in order to apply preventive measures.

A number of authors suggest the performance of those tests of hyper-susceptibility as a part of the pre-employment medical examination, since many industrial chemicals to which workers are exposed may develop similar responses to the hemolytic drugs.

Although very little is still known about individual susceptibility, the few elements available have to be carefully evaluated thus helping to place susceptible individuals in a job not affecting their well-being, i.e., protecting them against any potentially harmful exposure.

Our efforts have to be directed to obtain the maximum benefit of advances in instrumental analysis, in enzymology and in genetic studies.

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