TOXICOLOGY: INORGANIC

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As might be expected in these times of rapid accumulation of information and skills of investigations in the many disciplines that contribute to toxicology, the emphases on the various aspects of toxicology are changing. The current literature dealing with toxicology tends to move further and further away from the mere anecdotal accounting of case histories and statistical compilation of cases to more fundamental considerations as: attempts at the quantitation of body fluid and tissue levels associated with malfunction as manifest in signs and symptoms; studies on the kinetics of noxious ions, and the effect of various agents on the ebb and flow of such ions; and investigations on the biochemical alterations associated with the influx of noxious materials and the mechanisms involved in the development of pathologic changes. In clinical toxicology, a trend manifested for quite some time now becomes even more apparent in recent years; namely, the preponderance of inorganic poisoning as a result of occupational exposure as compared to such poisonings from other sources. As skills in detection and evaluation of slowly developing effects such as premature degeneration of tissues, lowered resistance to stress and disease, life shortening, etc., develop, one may expect that much of the emphasis of toxicology will tend to move further toward the study of the consequences of the accumulation of low levels of toxic materials in the general environment.

Arbitrarily, this review is limited to studies of the last three years, although reference to previous work is made when it is pertinent to do so. Of necessity, only limited aspects of each subject presented are covered. The literature was surveyed through July 1961.[1] For a perspective and summation of earlier work, the reader is referred to recently published texts: Browning—*Toxicity of Industrial Metals* (1); Deichmann & Gerarde—*Signs, Symptoms and Treatment of Acute Intoxications* (2); Stewart & Stolman—*Mechanisms and Analytical Methods* (3); Bensley & Jaron—*Handbook of Treatment of Acute Poisoning* (4); and Buchanan—*Toxicity of Arsenic Compounds* (5). The following reviews are concise, valuable sources for current information on *general toxicology* (6, 7); *isolation, identification and determination of poisons* (8); *industrial toxicology* (9); *toxicology of radioactive materials* (10); *pneumoconiosis and silicosis* (11); *pharmacology of the heavy metals* (12); and *bone seekers* (197).

AIR-BORNE POISONS

The inhalation of noxious materials in the course of occupational activity, or because of exposure to generalized air pollution, poses some of the most

[1] The survey of the literature pertaining to this review was concluded in July 1961.

[2] Abbreviations included in this chapter include: BAL (2,3-dimercaptopropanol); DTPA (diethylenetriaminepentaacetic acid); and EDTA (ethylenediaminetetraacetic acid).

important (from the viewpoint of numbers of people involved), challenging, and obstinate problems in toxicology today.

Dusts.—Of the air-borne toxic agents, the dusts are major offenders in producing disability, particularly as a result of occupational exposure. Formerly silica, asbestos, and beryllium oxide were the dusts implicated in the production of disease by induction of fibrosis in the lung. In the past few years, more and more dusts are being implicated as disabling fibrogenic agents: aluminum powder (13, 14); nepheline dust, mixed sodium, potassium, and aluminum silicates (15); aluminum phosphates, oxides, and hydroxides (16); china clay (17); talc, containing less than 0.5 per cent free silica (18); and graphite (19).

Silica.—Silicosis, unfortunately, is a common disease. Dreesen (20), in a report published in 1949, estimated that there were 110,000 persons in the United States with x-ray demonstrable silicosis. Trasko (21) reported in a five-year study (1950 to 1954) of 22 states some 11,000 cases of silicosis of sufficient severity to warrant disability payments.

The intriguing problem of the mechanism by which an apparently chemically-inert material such as silicon dioxide can react with tissue to produce striking and sometimes devastating pathologic changes continues to be the object of much interest and study. An historical account, comprehensive presentation, and critique of the theories as to the pathogenesis of the silicotic lesion have recently been presented by Schepers (22). This is an excellent source for much of the information that has been garnered over the years about the chemical and physical properties of silica and biochemistry of the silicotic lesion. Schepers points out that undoubtedly pathogenesis of the silicotic nodule involves physical, chemical and biological forces. He feels, however, that the tissue processes play a major role and the silica particle undergoes only minimal changes. He outlines the several phases in the development of the silicotic lesion and emphasizes that the critical end point, coincident with the onset of necrobiosis, occurs after the silica has been deposited in the tissue for weeks or months. At this time, a chain of events, as yet not clearly elucidated, disturbs a critical collagen-procollagen balance in the affected tissue, the result of which ultimately is fibrogenesis and collagen deposition.

An entirely different approach to the silicosis problem has been presented by Vigliani & Pervis (23, 24) of the University of Milan. They propose a theory based on an immunological mechanism. They are not, of course, the first to propose an immunological approach to silicosis but theirs is the most coherent theory presented so far, which ties together much recently developed information. The theory centers around the following facts: (*a*) The demonstration that the hyaline substance of the silicotic nodule is largely composed of gamma globulin (25, 26). (*b*) The silicotic nodule and regional lymph nodes contain many plasma cells. The deposition of the hyaline substance runs parallel to the appearance of plasma cells. (*c*) Serum gamma globulin levels are elevated in silicotic individuals (27). Immunoconglutinins,

agglutinating factor, and rheumatoid factor commonly seen in collagen disorders have been demonstrated at times in individuals with silicosis. (*d*) Injection of quartz activates the reticulo-endothelial system and enhances the production of antibodies, an anamnestic reaction. This is similar to the effect of endotoxic lipopolysaccharides.

Vigliani & Pervis suggest that the first link in the chain of reactions leading to the development of the silicotic nodule is the death of macrophages which have phagocytosed the quartz particles. They believe the disruption of the macrophages is due to activation of the first component of complement, or perhaps to activation of a specific esterase. From the dying macrophages, substances are set free which activate the reticuloendothelial system, antibody formation, and production of collagen fibers.

Gross (28) finds the immunologic approach to silicosis attractive and suggests that the concept could be applied to explain the disease-producing potential of other dusts (e.g., the massive pulmonary fibrosis seen at times associated with coal mine dust inhalation). In support of this, he has experimental evidence, namely, that massive pulmonary fibrosis was produced in guinea pigs administered coal mine dust and very low virulence tuberculin bacilli, each agent alone which is innocuous (29).

Although the prognosis of the worker simultaneously affected with tuberculosis and silicosis has been markedly brightened by recent advances in the therapy of tuberculosis (30, 31), the treatment of silicosis, per se, still leaves much to be desired.

Encouraged by the findings that tissue cultures of phagocytic cells could be protected against the toxic effects of silica dust by a number of orgainc bases and aluminum complexes, James *et al.* (32, 33) studied the effect of a number of drugs in inhibiting or retarding the development of silicotic nodules in the liver of mice after the silica dose was given intravenously. Drugs active in this respect were aluminum dextran complex, methylene blue, phenozene B-749, and a compound labeled 46--107. To produce significant effects, toxic levels of the drug were required. However, since massive doses of dust were injected, the hope is that in further studies a lesser degree of silica dust contamination might be overcome by lower doses, and hence less toxic doses of the drug.

Because silicosis is a slowly developing disease, the passing of a number of years is required before any prophylactic measures against silicosis can be properly evaluated in man. A sufficient length of time has now elapsed since the McIntyre prophylaxis regimen with aluminum powder was first shown fruitful in animals and trials in man begun (34, 35). Hannon *et al.* (36) recently reported their study, started in 1946 in workers exposed to silicon, wherein the McIntyre aluminum powder was administered by inhalation for six minutes, once a week, over a period of years. The conclusion is that the McIntyre aluminum powder decreased the usual progression of silicosis and that there are no harmful effects resulting from exposure to the aluminum dust. These findings are at variance with those of Kennedy (37)

who, after a four-year work-control study in pottery workers and in coal mines, concluded that the aluminum powder regimen was not sufficiently effective to be recommended for widespread application.

Air pollutants.—Although it is pretty well recognized that the air of many metropolitan and industrial areas is frequently contaminated with materials that can be harmful as well as irritating and, under certain circumstances, can be acutely life shortening, the detailed toxicology of specific offending pollutants is still quite fragmentary. Toxicological studies have so far been essentially inadequate for pinpointing the agents responsible for the acute symptoms observed in major smog disasters [i.e., in 1952 in London where 4000 people died over and above the expected death rate in a four-day "pea souper" (38)]. Even more difficult and challenging are the problems posed wherein efforts must be directed in delineating and evaluating the effects of prolonged exposure to low-level concentrations of mixtures of noxious materials: effects which are difficult to discern because they are slowly developing and are often only manifest in the exacerbation of ills and disabilities associated with the progression of life.

Ozone.—Ozone has been implicated as a toxic agent in atmospheric pollution, particularly in Southern California where meterorological conditions favor an oxidizing type smog. Although this substance has been shown to be toxic in animals and produce symptoms in man under experimental conditions, there is no clear cut evidence as yet that any disability has been produced in people as a result of exposure of this material in the environment. Severe ozone intoxication, however, has been reported in three cases as a result of exposure from the use of inert-gas-shielded arc welding processes (39).

A number of workers [Haagen-Smit & Fox (40); Littman, Ford, & Endow (41)] explain the presence of appreciable levels of ozone in the atmosphere over the Los Angeles basin (the highest known concentration of ozone on the earth's surface, about 4 p.p.m. in mid-day) on the basis of the interaction of nitrogen dioxide with hydrocarbons and their oxidizing products from automobile exhaust under the influence of sunlight.

Mittler *et al.* (42) have done extensive studies on the toxicity of ozone. They observed that repeated exposures of rats to 2.4 p.p.m. of ozone (levels found in the Los Angeles atmosphere) induced hemorrhage and edema of the lungs. Continuous exposure of mice to this level resulted in death within ten days. It is significant that the exposure of the human being to levels of ozone of this order produced symptoms rather quickly (43). A two-hour exposure at a level of 2 p.p.m. resulted in throat and mouth dryness, loss of mental ability to concentrate and absorb thoughts, substernal signs of a constrictive nature, and a continuous sensation of odor. No eye irritation or nausea was incurred.

Tissue damage, characterized pathologically as chronic bronchitis and bronchiolitis, was demonstrated by the Stokinger group (44, 45) in rats following repeated inhalation of ozone at concentrations only two to three

times greater than that currently reported in the atmosphere. The terminal air-ways of the lungs were thickened and air passages narrowed. Fibrosis and eventually emphysema developed.

The demonstration of development of a tolerance to ozone (46, 47) may explain the discrepancies between the findings under the experimental conditions described above and the lack of symptoms attributable to ozone in the Los Angeles population, despite the frequent presence of appreciable ozone levels in the atmosphere above that city. In this context, it is interesting to note that the ozone concentration in pressurized cabins of airplanes flying at 25,000 to 40,000 feet is 1 to 2 p.p.m., and occasionally as high as 4 p.p.m. (48). At these altitudes, there is a stratum of ozone of such concentration that when the air is compressed to an atmospheric pressure corresponding to 8,000 feet of elevation, the ozone content is 1 to 2 p.p.m.

Oxides of nitrogen.—According to Gray (49), nitrogen oxide, nitrogen dioxide, and nitrogen pentoxide are the compounds of significance, among the possible nitrogen oxides, as air pollutants.

Nitrogen oxide is relatively nontoxic. Levels of 310 p.p.m. have not been found toxic to animals after eight hours of continual exposure (50), and there have been no cases of nitrogen oxide poisoning reported in humans.

Nitrogen dioxide is considerably more toxic than nitrogen oxide, but it is difficult to quantitate this toxicity precisely. A syndrome termed "silo filler's disease," occurring in farmers during work in silos, has been postulated to be a form of NO_2 pneumonia (51).

Results of toxicity studies on various species of animals vary considerably from investigator to investigator. Death occurred in some species after exposure to 55 p.p.m. for two to three hours (52). In studies by Gray *et al.* (53), animals were able to withstand 100 p.p.m. for $5\frac{1}{2}$ hours before death occurred. Tollman *et al.* (54) concluded in their study that NO_2 could be inhaled at air concentrations in excess of 100 p.p.m. for four hours per day for many weeks before fatal results would occur. Gray suggests a possible explanation of these conflicting results; that in some of the studies the gas mixtures used contained varying, but undetermined, amounts of contamination with NO.

In the presence of ozone and NO_2, N_2O_5 can be found in the atmosphere. The toxicity of this compound is also controversial. Diggle & Gage (55) found that pulmonary edema was produced in rats after four hours of exposure at levels of 2 p.p.m. Stokinger (46) has been unable to confirm these results and believes that N_2O_5 is relatively innocuous.

The ubiquitous association of the oxides of nitrogen and ozone as atmospheric pollutants and in mixtures used in experiments has contributed to the confusion as to the toxicity of these materials. Thorp (56) is an exponent of the school of thought which holds that the toxicity of such mixtures is largely due to nitrogen oxides and that ozone is essentially nontoxic. Diggle & Gage (55) believe that nitrogen pentoxide and ozone are synergistic in their toxic effects, and recently studies of Stokinger (46) and Svirbely (47)

indicate that the presence of the nitrogen oxides does not significantly affect the toxicity of ozone.

Oxides of sulfur.—Much like the situation for ozone in oxidizing smog, it has been frequently postulated that in a reducing-type fog oxides of sulfur (SO_2 and SO_3 dissolved in droplets to form H_2SO_4) are the principal offenders in production of irritation and disability (57). There is ample evidence to show that these oxides will damage forests, particularly conifers (58) to the leeward of smelters, and at even very low levels damage many species of plants (59). The concentration of these materials in the atmosphere (i.e., on the order of 1 p.p.m.), however, never reaches the levels required to produce the toxicity demonstrated in experimental studies (60). In order to explain the physiologic and pathologic reactions which are apparently disproportionate to the air concentrations of these noxious materials, it has been suggested that the adsorption of gas molecules on particles settling on the mucous membranes of the respiratory tract might produce high concentrations locally—concentrations which are much higher than those measurable in the air (60)—and thereby produce effects.

Amdur (61) has investigated the synergistic action of SO_2 and H_2SO_4 mist as a possible explanation for the irritation potential of fog. She found that acid mist particles of 0.8 μ mixed with SO_2 resulted in a toxic mixture that was more potent than suspensions of the acid mist or the SO_2 alone. She also showed that a sodium chloride aerosol considerably enhanced the potential toxicity of such mixtures.

Balchum and co-workers (62), in a series of studies using $S^{35}O_2$, have recently contributed detailed information on the dynamics of sulfur dioxide inhalation. Sulfur dioxide is rapidly absorbed from both the upper and lower respiratory tract, even when inhaled in low concentrations (1 p.p.m.). It is distributed to all tissues, including the brain, and can be detected in the trachea and lungs as little as one week after exposure. It travels in the blood combined with plasma proteins and is excreted solely in the urine.

Carbon monoxide.—Like the other constituents which pollute the atmosphere, the contribution of carbon monoxide to discomfort or disability has not as yet been satisfactorily evaluated. For every part per million of CO which reaches the lungs, it is calculated that 0.16 per cent of the body's hemoglobin is inactivated (63). Maximum figures of 93 p.p.m. of CO have been recorded in the atmosphere, but more usual figures correspond to between 4 and 8 p.p.m. Cigarette smoking adds to this level, and in urban smokers up to 8 percent of the hemoglobin may be inactivated. Below levels of 5 per cent carboxyhemoglobin, their are usually no complaints; above 20 per cent, the symptoms are moderate to severe; and concentrations above 50 per cent may produce coma and death. Symptoms are those associated with anoxia, fatigue, dizziness, and headache. At higher levels of exposure, neurological signs are apparent: ataxia, twitching, and in time, coma.

Gilbert & Glaser (64) recently reported a case diagnosed as chronic carbon monoxide poisoning in a policeman who had exhibited unexplicable

neurological and psychiatric signs and symptoms for a period of years. Finally carefully questioning revealed exposure to high levels of CO in the course of his duties. His blood was found to be 20 per cent saturated with CO. After a period of time away from the job, his symptoms cleared. This case is of interest because it illustrates a contention by Loeper (65) that carbon monoxide may persist in the body for long periods—not just for days but for as long as three weeks. The mechanism of this is not clear but may represent the binding of CO to tissues as in the form of muscle myoglobin. The slow release of the material back into the blood could then well lead to a type of chronic poisoning. The problem has been considered in detail in a monograph on the subject by Grut (66). Not all authorities agree that chronic carbon monoxide poisoning occurs (67, 68); but indeed if Loeper is right, conceivably in times of unusually high air pollution the gas could accumulate in the body and produce symptoms.

METAL POISONS

Arsenic.—Among the more pressing problems of arsenic poisoning at the present time are those associated with inhalation of arsine, largely because this is the principal source of arsenic poisoning today. Poisoning has occurred insidiously as the result of exposure to fumes formed by the action of water or acid on traces of arsenic which have occurred as an unsuspected contaminant in other metals. Cases of arsine poisoning have been reported recently in the refining of tin and lead (69), in processing of zinc metal (70, 71), and in the cleaning of tanks (72). Poisonings with arsine have been of dire consequence not merely because arsine is such a powerful hemolytic agent but because to this day there is no adequate therapy for counteraction of its effects. The use of dimercaprol has been uniformly disappointing. It is possible that thiols or dithiols capable of penetrating red cell membranes might bind arsine intracellularly and thereby prove therapeutic. Historically, the demonstration of the relationship of toxicity of arsenic to thiol groups in tissue was the first instance of the interpretation of the selective action of a toxic agent in chemical terms. Since then, several enzyme systems have been shown to be affected by arsenic (i.e., α-glycerol phosphate dehydrogenase, lactic acid dehydrogenase, and cytochrome oxidase). Potentially, a host of enzyme systems should be susceptible to arsenic in view of the propensity of metals to react with such ubiquitous ligands as sulfhydryl, carboxyl, amino-imidazole, and phosphoryl groups not only present on the enzymes themselves but on cofactor actuators and coenzymes associated with enzyme systems. The demonstration of such interactions *in vitro* does not necessarily mean that these reactions are significant in determining the effects of arsenic in the body tissues. However, one interaction of arsenic, that with the cyclic dithiol lipoic acid, which is a critical cofactor in the pyruvate-oxidase system in the brain, undoubtedly plays a role in the production of neurologic signs and symptoms since other interferences with this system such as induced thiamine deficiency, produce the same signs and symptoms. It is postulated that

the therapeutic efficacy of dimercaprol is related to the fact that the five-membered dimercaprol-arsenic ring is more stable than the six-membered lipoate-arsenic ring.

Vallee, Venner, and Wacker have recently prepared an excellent review on the toxicology and biochemistry of arsenic (73). Among many things, they present an interesting opinion, namely, that while arsenic may be a factor in the predisposition to skin cancer, there is no valid basis for assuming that this element plays any role in the causation of other types of cancer.

Boron.—The boranes are the boron compounds of current interest in that they are now being used extensively for high energy fuels. Until recently, their toxicology was essentially unknown. In the past few years, intensive reserarch has been carried out and the toxicity of these compounds has been reported in a large number of studies.

Diborane is a gas at ordinary temperatures, and hence its potential hazard is largely through inhalation. The symptoms and pathology following exposure to the gas have been related to the respiratory system (namely, dyspnea, cough, light-headedness, and a sense of constriction in the chest). At high dose levels, neurologic symptoms (headache, dizziness, vertigo, chills, and occasionally fever) have been reported (74).

In animal studies (dogs and rats), there were no deaths seen after prolonged exposure to 1 to 2 p.p.m. Deaths began to occur at the 5 p.p.m. level after exposure of six hours a day for five days a week. At levels of 300 p.p.m. for fifteen minutes, death occurred rapidly. Pathology was limited to the lungs, namely edema and hemorrhage (75).

Diborane hydrolyzes rapidly to boric acid and hydrogen. The amount of boric acid produced is out of proportion to the toxicity seen. Damage undoubtedly occurs from the heat produced by the exothermic reaction and from some of the diborane which is absorbed before it is broken down, and perhaps from polymerized boranes which are formed in small quantities.

Pentaborane is a liquid at ordinary temperatures. Decaborane is a solid. Both are readily absorbed when taken into the body through all portals of entry: by inhalation, by digestion, and through the intact skin and mucous membranes.

At 0.2 p.p.m. pentaborane aerosols administered by inhalation for six minutes to rats, there was loss of appetite, weight loss, apathy, docility, decreased physical activity, and muscle tremors (75). At levels of 1 p.p.m. administered repeatedly for five hours a day, death will result in a small proportion of animals by four weeks. At 3.5 p.p.m. five hours per day, animals began to die by the end of the fourth day. Levels of 10 p.p.m. were acutely toxic. The signs observed were of a neurological nature (tremors, convulsions, and coma, passing into death). Gross pathological findings were slightly darkened adrenals, congested and fatty levels, congested lungs, and ejaculated semen. Corneal opacities developed within a short time after exposure to the boranes.

The administration of decaborane by aerosol inhalation produced similar

effects and pathology, but the effects were delayed in onset and in nonlethal situations persisted for longer periods of time. Svirbely (76) found the LD_{50} for decaborane aerosol in mice exposed by inhalation for four hours to be 36.5 p.p.m., and for 24 hours' exposure to be 25.7 p.p.m.

The evaluation of the extent of absorption of the boranes as a result to exposure to low levels until recently has not been possible. Clinical effects are delayed in manifestation; decaborane and pentaborane do not appear as boron in the urine until some time after exposure. Liver and kidney function tests are of limited value since there was no direct correlation between manifest damage to the tissues and the extent of exposure. A simple means for demonstrating the absorption of low levels is particularly necessary, since the effects of boranes are cumulative, and it is desirable to remove an individual from exposure as soon as build-up is detected. Miller *et al.* (77) have developed a procedure for the assay of serum borane levels. Using this procedure, they have been able to develop a relationship between serum borane levels and clinical signs of the toxicity for decaborane. Detectable serum borane levels appeared in animals 15 minutes after intraperitoneal injection of acutely toxic doses of decaborane. Levels remained elevated for three to four days after moderate exposure and up to seven days after severe exposure. Daily administration of doses below those to avert toxicity resulted in a gradual build-up and eventual death. Rapid penetration of decaborane through the skin was detected through the elevation of serum borane levels in humans, even though the decaborane was allowed on the skin for only a few minutes.

Cadmium.—Much of the current toxicological interest in cadmium is on the effects of repeated inhalation of low doses of dust or fumes of cadmium compounds. It has been well established for years that cadmium can be acutely toxic, and that exposure to high concentrations of cadmium compounds dispersed in air can bring about distressing respiratory symptoms: marked dyspnea, uncontrollable coughing, severe chest constriction, etc., These symptoms are often followed in a few days by death from anoxia, attributable to massive pulmonary edema, or from intra-alveolar hemorrhage. However, it is only recently that it has become recognized that cadmium can be a systemic poison as well as a pulmonary irritant when there has been repeated exposure to low doses—doses passing unnoticed because they are not sufficient to bring about immediate respiratory reaction.

Princi (78), in a study of workers exposed to cadmium in a smelting operation, found no evidence of disability related to such exposure and attributed the lack of demonstrable cumulative effects to the fact that cadmium turned over rapidly in the body. At about this same time, Hardy & Skinner (79) found there has been evidence of poor health in workers engaged in fabricating cadmium-plated parts, but did not have sufficient information to make any definitive correlation with cadmium exposure. Prior to and shortly following these reports, evidence began to accumulate from studies of European plants strongly indicating that protracted exposure to cadmium

could markedly influence well-being (80 to 84). The difference in the findings undoubtedly is due to the different exposure conditions in the various areas.

The results of a recent follow-up study (85) on men exposed to cadmium oxide fumes should remove all doubts about the hazards from exposure to cadmium oxide. In 1953, Bonnell (84) surveyed a group of 100 men exposed to cadmium oxide fumes from cadmium copper alloy operations. At that time, 23 cases of chronic cadmium poisoning were uncovered. Four years later, when this follow-up study was carried out, 24 new cases were found. Four of these men had not been exposed since 1953 and at that time they had been normal. Diagnosis was made on findings of emphysema and a characteristic proteinuria, although not all cases showed both. Friberg (86), in his survey of chronic cadmium poisoning, notes a number of other signs and symptoms commonly seen, which were not reported by the British, such as anosmia, ulceration of the nasal mucosa, yellowing of the teeth, nephrolithiasis, and occasional signs of liver damage. The pulmonary emphysema seen by Bonnell *et al.* was almost uniformly progressive in all cases. Death, when it occurred, most often was a consequence of the emphysema or complications thereof. According to Bonnell, cadmium emphysema is different from the chronic hypertrophic variety which follows long-standing bronchitis or bronchial asthma. Cadmium emphysema, while widespread, shows a narrow zone of normal lung tissue underneath the pleura and no emphysematic bullae in the periphery (87).

The proteinuria is characteristic (88) in that a protein of low molecular weight (20,000 to 30,000) is excreted. It is readily distinguishable by simple clinical laboratory tests. It is not precipitated by heat or by picric acid but can be brought down with 25 per cent nitric acid, 25 per cent trichloroacetic acid, or 3 per cent sulfosalicylic acid. The quantity excreted is relatively small—0 to 4 g. per liter (89). The cause of proteinuria has not as yet been determined. The protein may be formed elsewhere in the body other than the kidneys and may leak across the glomerulus by virtue of its low molecular weight. In this connection, a report by Lawford (90) of a low molecular weight protein seen after the injection of cadmium, as well as other metals, is of considerable interest. On the other hand, the protein excretion may be related to renal damage which has been shown to occur (91).

Urinary assay for cadmium is of value only as a confirmation of cadmium absorption. It has not been possible to demonstrate any relation between urinary cadmium levels and the length of time and extent of exposure or the severity of the poisoning.

The anemia, when it does occur, has been shown to be related to an increased rate of destruction of red cells (92). It is pertinent to note that cadmium is carried in the blood in the red cell combined with hemoglobin (93). Part of the lowered red count may be due to a decrease in hematocrit as a result of the progressive expansion of plasma volume which appears in time with the disease (94).

Cadmium accumulates chiefly in the liver and kidneys and to a lesser

extent in the thyroid, pancreas, and testes (95). In these studies by Friberg, tissues taken from individuals who had been out of exposure for as long as nine years showed appreciable levels of cadmium. Friberg interprets this as indicative of a very slow turnover of cadmium from the body.

Parizek & Zahar (96) found that small doses of cadmium administered to rats produced selective damage to the testes. Parizek (97), in following up this observation, found that large doses of zinc salts injected simultaneously with the cadmium afforded complete morphological protection to the testes. Gunn *et al.* (98) have recently found that the damage to the testes, which consists of degeneration of the seminal first tubules, is irreversible but that the interstitial cells quickly regenerate and eventually produce antigens. These workers demonstrated that the protection afforded by the zinc was temporary.

Both Ca EDTA and dimercaprol have been found to enhance cadmium excretion markedly. Friberg (99,100) noted in his studies that administration of each of these drugs to chronically cadmium-poisoned animals produced marked kidney damage and cautions against their use. However, it is not surprising that he observed the renal changes he did. The doses of Ca EDTA (500 mg to over 1000 mg per day) are tremendous, equivalent to 25 to 50 g per day for a human. Foreman *et al.* (101) have shown that doses of Ca EDTA of such magnitude do indeed produce kidney damage in a few days. There is no direct relationship between the size of the dose of Ca EDTA and its efficacy in hastening excretion of metals (102). Doses of the drug at levels of 5 to 10 mg per day, in all likelihood would have been just as effective as the 500 mg dose. Moreover, repeated courses of treatment interspersed with periods of rest are the recommended mode of administration of EDTA. Cotter (103) has had good response in three cases of chronic cadmium poisoning after giving Ca EDTA by mouth. One would expect intravenous administration to be much more effective, since the drug is poorly absorbed from the gastrointestinal tract (104). It appears that one could be well justified in using Ca EDTA in cadmium poisoning provided it is used judiciously and with proper follow-up (101).

Chromium.—Current interest in chromium from a toxicological viewpoint lies in its apparent carcinogenic propensity. Individual observations and epidemiological studies have presented strong evidence indicating that workers in chromium experience a higher risk to cancer of the lungs than do individuals comprising the general population (105 to 109). These observations have stimulated a number of attempts (110 to 114) to induce carcinoma in animals through the introduction of various chromium compounds. These were essentially futile until recently, when Hueper & Payne were able to demonstrate conclusively the deliberate induction of sarcoma and carcinoma in mice and rats. In his first experiments, Hueper (115) implanted the insoluble residue from a water-extracted chromite ore into the thigh and pleural cavities of rats. After two years, there was a development of squamous cell carcinoma concurrently with sarcoma in the lungs of 2 of 25 rats and

3 fibrosarcomas in the thighs of 31 rats. Hueper attributes these successful results, as compared to the failures of others, to propitious combination of chemical and physical properties of an injection mixture which eliminated the acute toxic effects of highly toxic hexavalent chromium and yet allowed the slow release of this active form of chromium at concentrations sufficient to produce chronic irritation. Payne (116) was able to reproduce these studies and demonstrated further that the carcinogenic activity of chromium was direct rather than secondary, since chromium salts injected with a powerful carcinogen (3,4-benzypyrene) inhibited rather than promoted the induction of malignancy. Later, Hueper & Payne (117) collaborated in a study wherein pure chromium compounds (calcium chromate and chromium trioxide) were shown repeatedly to produce carcinoma.

Iron.—Iron compounds have generally been considered of so little consequence as toxic agents that they are usually not included in standard text books of toxicology. Recent reports, however, should draw attention to this deficiency (118, 119). Sisson (120) reports on acute iron poisoning in children. He points out that the widespread use of hematinics has resulted in the availability of a highly palatable and tempting source to children. Doses of as little as 3.3 g of iron sulfate have been reported to cause death in small children (121). The margin of safety between therapeutic doses for adults and toxic doses for small children appears to be small. Sisson emphasizes that the death rate from this form of intoxication is higher than that from acetylsalicylic acid.

Contrary to what might be expected from a consideration of mucosal block theory, large doses of iron are absorbed rapidly through the stomach and large and small intestines. Serum iron rises roughly in proportion to the dose absorbed and may reach as much as ten times normal. The overwhelming concentration of iron in the plasma rapidly exhausts available transferrin and circulates in a loose combination with albumin and as free ion. Its activity in this form is apparently similar to that of ferritin in that it produces vasodepression leading to hypotensive shock which, in severe cases, develops into cardiovascular collapse and eventual death. An increase in coagulation time results because of a development of hypoprothrombinemia and a decrease in fibrogen activity (122). Sisson has found the use of Ca EDTA valuable in two trials in which the levels of serum ionic iron in the plasma were markedly reduced (123).

Fahey and co-workers (124) believe another chelating agent (trisodium calcium diethylenetriaminepentaacetic acid, Ca DTPA) to be much more effective than Ca EDTA in hastening the excretion of iron, even iron bound to tissue. They were able to induce the excretion of iron up to 109 mg per day in a patient with hemochromatosis following the use of this drug.

Of great current interest is the confirmation by Haddow (125, 126) that Imferon, an iron dextran compound, will, on repeated subcutaneous injection, induce sarcoma in mice. Weekly doses, as small as 0.05 ml, of Imferon injected subcutaneously induced the malignancy. In considering the implication of the use of this preparation in man, Haddow emphasizes that the

carcinogenic effect is determined mainly by the absolute amount administered and only secondarily, if at all, by the relationship of dose to body weight. He urges caution in the use of parenterally administered preparations of iron.

In a study designed to investigate iron overloading phenomena in animals, Goldberg, Martin, & Smith (127) set up groups to observe the development of sarcoma after repeated injections of various iron compounds. They were not able to induce sarcoma in mice after repeated injections of Imferon, even when using the Chester Beatty strain of mice that was used by Haddow. They did observe sarcomata in rats, as did Richmond in 1959 (128). They found a relationship between the amount of iron injected and the frequency of occurrence of sarcomata. They feel that their data indicate that there is a threshold level below which sarcoma does not develop—a level well above that which would come about from a repeated therapeutic application of Imferon.

Lead.—The literature on lead poisoning is perennially voluminous. In 1922, a list of publications appeared in which 2000 references were cited. A review on the pharmacology of lead in 1934 stated that 10,000 references could be found [quoted by Passow *et al.* (12)], and the present time proves no exception. A whole volume could easily be written, if one wished to present a complete evaluation of all aspects of the toxicology of lead.

Recent interest in lead is high primarily because of two developments in the past few years. One is the introduction of chelating agents which appear to be useful in the diagnosis and treatment of lead poisoning. The other is the increase in case findings of childhood lead poisoning attributable to the increased awareness of physicians to this possibility, undoubtedly stimulated by the publicity given by Dr. Huntington Williams to pica involving lead in paint and plaster in old houses as a possible source of lead poisoning. Byers (129), in his review on lead poisoning, points out that Mellins & Jenkins (130) reported 21 cases in a Chicago hospital in one year, after there had been no cases diagnosed for many years. Similarly in New York, the number of cases diagnosed and reported to the Board of Health rose steadily from 1 in 1950 to 80 in 1954 (131).

Among the chelating agents which have recently been studied for their effectiveness in hastening excretion of lead from the body and found promising are Ca EDTA (132), Ca DTPA (133), and DL-penicillamine (134). Penicillamine is not nearly so effective in removing lead from the body as DTPA or EDTA, but it is of interest because it can be used orally and it is practically nontoxic. DTPA is probably as effective as EDTA but has not as yet had extensive clinical trial. EDTA has been very widely used. Several reports reviewing the current status of chelating agents in the treatment of heavy metals have recently appeared (132, 198, 199).

Calcium EDTA has been found very helpful in the diagnosis of lead poisoning, particularly in cases where urine levels were not very high. A provocative dose, ranging from 0.3 g to 2 g of EDTA, is given intravenously (135 to 140). Twenty-four hour urine specimens are collected and assayed

for lead. Generally speaking, an outpouring of 1 mg in 24 hours is considered indicative of an excessive body burden of lead.

One of the first questions that arises in a consideration of the use of chelating agents concerns the actual necessity for a definitive therapeutic agent which can effect the removal of lead from the body. There is a school of thought which holds that except for supportive therapy in the alleviation of acute symptoms, an individual with lead poisoning is just as well off not treated. This approach to the lead poisoning problem is based upon the reasoning that lead poisoning is a self-limiting disorder, in that removal of the individual from exposure to lead will in time result in the disappearance of symptoms and the restoration of well-being. The disappearance of toxicity comes about because the bulk of lead in the body eventually becomes tied up in the skeleton, wherein supposedly it is rendered harmless. The effectiveness of EDTA in accelerating the disappearance of symptoms and signs of toxicity has contributed significantly towards dispelling this more conservative approach to the management of lead cases.

Actually, "dormant" lead in the skeleton is not entirely harmless. It has been reported, particularly in children (141) but also in adults (142), that the symptoms of lead poisoning recur in individuals who have had excessive absorption of lead and then at some time later undergo stress such as febrile illness, severe malnutrition, some chronic diseases, or severe injury. For this reason, alone, it would be desirable to rid the body of as much lead as possible, if the means for doing this are available, convenient, and harmless if done properly.

The greatest need for improvement of therapy of lead poisoning lies in childhood cases, for it is here that the effects of lead poisoning are most distressing. Chisolm & Harrison (143) studied the effects of treatment with BAL and EDTA in children with varying degrees of severity of lead poisoning. They concluded that the administration of Ca EDTA did not significantly affect mortality but that the drastic consequences of lead encephalopathy in survivors were markedly diminished. Byers (129) in his study came to essentially the same conclusion. The intravenous use of urea for the reduction of increased intracranial pressure in lead encephalopathy in conjunction with Ca EDTA, as suggested by Katz (144), may well prove to be life-saving therapy in this distressing disorder.

Although it has been shown repeatedly that in adult cases symptoms and disability disappear in time after the individual is away from exposure, the use of EDTA has been found to hasten the disappearance of abnormal findings such as colic (145), muscular weakness and tremors (137), anemia (146, 147), basophilic stippling (148), coproporphyremia (129, 149) but not protoporphyremia in the red cell (129), and bone marrow pathology (147, 150).

Calcium EDTA by all routes of administration, intravenous, intramuscular (129), subcutaneous (143), oral (151, 152), and by aerosol inhalation (153), increases the urinary excretion of lead. Except for oral administration, which is not very effective, all routes bring about essentially the same re-

sponse. The amounts excreted depend upon the body burden, and the period elapsed between the time when the drug is given and the metal has entered the body. The sooner the drug is administered, the more effective it is. Up to 20 mg of lead (139, 154) has been excreted in a single day and as much as 70 mg in seven days of treatment (139).

Seven has summarized the reports on the side effects, and toxic manifestations of Ca EDTA. He discussed these at a recent symposium on chelating agents (155). Of all of these, the most significant is nephrotoxicity. Several deaths occurring while patients were receiving large doses of EDTA showed renal tubular lesions at autopsy (156 to 159). Foreman *et al.* (101) were able to demonstrate that tubular lesions could be repeatedly produced in rats after prolonged dosage at various levels of EDTA. They found these lesions to be reversible. These findings suggested that EDTA is best given in short courses with interspersed periods of rest.

The development of effective agents for mobilizing metals from the body, has raised many questions about the disease process of metal poisoning itself; many of which have been considered in the past, but are now being brought into focus with increased interest and need for solution. For instance, it has now become pertinent to know more precisely the size of the body burden of metal in an individual so that it might be possible to establish the proportion of metal in the body that is mobilized by treatment.

Chisolm & Harrison (143) estimate that during the acute stage of poisoning there was between 20 to 100 mg of lead in the soft tissues of various children in their study. Probably an equal amount is in the skeleton (160). If their estimate is correct, then a series of treatments with Ca EDTA could essentially remove the lead from the soft tissue in time. Even more desirable is information on metal levels that are associated with tissue damage in specific organs. Other information on the behavior of metals in the body, which have now become cogent, are the transfer rates of metal ions from one body compartment to another (i.e., from tissue cells to interstitial fluid to the blood to intraluminal cavities, etc.), and the effect of the introduction of chelating agents into the body on these rates. If one could establish the rate-determining step in the course of events, then attempts could be directed toward the design of a chelating agent with specific capability for hastening transfer across the particular membrane involved.

There is still considerable ambiguity as to the optimum dose of chelating agents, size, frequency, rate of injection, and mode of administration—— even of the most widely used drug, Ca EDTA. The primary limitation on the size of dose that can be given is the nephrotoxicity of the drug. Because of this, an upper dose of 50 mg per kg per day for five days with a minimum of two days' rest was recommended. This would appear to be the optimum dose. However, a number of workers have shown that there is no simple relationship between size of dose, and effectiveness, and that smaller doses have a greater relative efficiency. Bell (137) found that 3g doses brought forth only 3.3 times as much of an increase in lead excretion as did a 0.1 g dose. Leckie *et al.* (102) found that a 4 g dose was no more effective than a 2 g

dose and that a 4 g dose brought forth only 2.5 times as much lead as did a 50 mg dose. Moreover, the rate at which a dose is given varied the effectiveness of the drug. Giving the dose over a six-hour period achieved double the urinary lead excretion of the same dose given in one hour. Because of the rapid turnover time of the drug (1½ hours) in the body, one would expect that divided doses over the day would give a more constant blood level than the same dose given in a single injection. Actually, a regimen of a single dose per day has been found to be as satisfactory as any other scheme. This is all puzzling, and may be cleared up when more is known about the behavior of the lead chelate in the body. A possible explanation of the relatively greater efficiency of lower doses is that somewhere in the transport chain there is a membrane whose transfer potential for Ca EDTA is saturated at low concentrations.

Mercury.—Although the toxic potential of mercury has been widely recognized for years, and the means for control of inadvertent exposure are known and simple to apply, many cases of mercury poisoning are still being reported (in the manufacture of electrical brushes (161), of thermometers (162), in laboratories (163), and in the home (164)).

It is only recently that a satisfactory study of the kinetics of mercury in the body has been reported. Rothstein & Hayes (165) followed the behavior of mercury given intravenously and intramuscularly as $Hg^{103}NO_3$ to rats for as long as 95 days. Clearance from the body showed three distinct phases. The first involved 35 per cent of the dose, having a half-time of three to four days——probably representing the build-up (in this case, very rapid) and subsequent excretion from the liver into the feces and translocation into the kidneys. The second component, involving 50 per cent of the injected dose, had a half-time of 30 days, and was the net resultant of the accumulation of the ion in the kidneys, and excretion from that organ into the urine. The third component, involving 15 per cent of the dose with a half-time of approximately 100 days, is determined by excretion from the kidneys. By the end of one week, essentially all of the material (85 to 95 per cent) that is in the body is stored in the kidneys.

This affinity of the kidney tubular tissue for mercury has not as yet been explained. A similar study using various amounts of carrier mercury would be most valuable to show the extent to which the damaged kidney alters the ebb and flow of the metal. Studies using tracers carried out by others verified this distribution pattern in general (166, 167); and also showed that, as opposed to trivalent mercury ion, a number of organic mercury compounds readily pass the blood-brain barrier and accumulate in the brain.

In the blood, less than 1 per cent of the mercury is filterable. Of the bound mercury, one-half is associated with the red cells, and the other one-half is bound to albumin in combination with sulfhydro groups (168). Inhaled mercury is oxidized in the lungs and passes with a half-time of seven hours, across the alveolar membranes into the blood as ionic mercury (168).

Demis & Rothstein (169) have recently provided information on the interactions of mercury, at the tissue and cellular level. They demonstrated

that mercury first combines with functional groups on the cell membrane—probably thiol groups, since mercury forms very stable mercaptides, and does so in preference to reactions with other ligand groups (170). Subsequently, the mercury ion slowly enters the interior of the cell. When excised rat diaphragm was exposed to mercury ion, the rate of uptake was rapid for about 20 minutes, and then proceeded at a much lower rate. In the first phase, the uptake of glucose by the muscle was inhibited; and in the second phase, respiration became progressively inhibited. On the addition of BAL or cystine, the inhibition of glucose transport was reversed, whereas that of respiration was not. In homogenates where the cell membrane is broken down, the inhibition of respiration occurred almost immediately on addition of the metal. Similar effects have been observed on the membranes of the epithelial cells of the jejunum. Immediately following the addition of the metal (as $HgCl_3$), rapid responses are observed in the electrical potential across the intestinal wall, the loss of cellular potassium, and the cessation of glucose uptake (171). After a delayed period, other functions are inhibited. A detailed discussion of these effects is presented by Passow *et al.* (12).

Diverse reports on the effectiveness of various chelating agents in the treatment of both chronic and acute mercury poisoning have appeared. Longcope & Luetscher (172), Raskam *et al.* (173), Bell *et al.* (174), and Hadengue *et al.* (175) have found BAL to be useful in both chronic and acute cases. Hadengue & Bell found no value in the use of Ca EDTA. Woodcock (176) recommends Ca EDTA in preference to BAL, on the basis of his experience in the use of both. Recently, D-penicillamine (177) and one of its analogues (N-acetyl-DL-penicillamine) have been observed to be highly effective in reducing the mortality of rats from mercuric chloride (178). Both of these drugs have the advantage of oral administration, and very low toxicity.

Nickel.—Systemic poisoning from nickel salts, is almost unknown from industrial exposure or otherwise. However, a nickel dermatitis, "nickel itch," is of consequence; and recently has been reported to be increasing in frequency among the industrial dermatoses and from nonoccupational exposure (179). Both Ca EDTA and sodium diethyldicarbonate (180) have been recommended as being effective in preventing the appearance of lesions, if these drugs are applied in an ointment within two hours after the nickel compound has been in contact with the skin.

The principal hazard from nickel is in the form of nickel carbonyl, largely because of the development of cancer of the lungs and nasal cancer which appear to result from exposure to this material. Epidemiological evidence collected by Doll (181), Morgan (182), and Williams (183), strongly indicates that exposure to nickel carbonyl increases the risk of carcinoma in these organs. Morgan, however, believes that the risk has largely been eliminated, since improvements in plant processes have been instituted. Sunderman *et al.* (184) were able to implicate nickel carbonyl conclusively in induction of pulmonary cancer in rats. Rats given a heavy exposure, as

well as others given repeated sublethal exposures for one year, began at the end of one year after cessation of their exposure to show various types of cancer of the lungs. Sunderman and his group have also followed the effect of chelating agents on excretion of nickel carbonyl. Calcium EDTA was of no value (185). The dialkyldithiocarbamates (186), however, gave complete protection against 100 per cent lethal doses of nickel carbonyl in mice. Diethyldithiocarbamate was subsequently used in 11 persons who had been acutely exposed to the gas (187). Toxic symptoms were rapidly relieved and there was a considerable increase in urinary nickel after the drug was administered.

Selenium.—As compared to the age-old poisons such as mercury, lead, etc., the recognition of selenium as a source of poisoning is relatively recent. As a consequence, much of the fundamental toxicology and biochemistry of the metal is still being developed.

On the basis of a study which showed a very small effect of selenite on the combination of several sulfhydryl enzymes, Tsen & Collier (188) conclude that mercaptide formation is an unlikely mechanism in explanation of the toxic effects of selenium. They found it is more likely that the toxicity comes about from a selenium-catalyzed oxidation of such cofactors as glutathione, coenzyme A, and dihydrolipoic acid, and the consequent disturbance in intermediary metabolism.

Only recently has it been shown that selenium is an essential trace metal. It has been demonstrated to be a necessary requirement for optimum growth in chicks. It is an essential element in the diet of rats, and in mice as well, in that the lack of a dietary agent identified by Schwarz (189) as Factor 3, an organic compound containing selenium, results in liver necrosis in rats and multiple degeneration (heart, liver, kidneys, and muscle necrosis in the mouse (190)), as well as exudative diathesis in chicks (191). The addition of selenite or selenium in organic form will prevent these manifestations in all three species (189, 190, 192, 193). Selenium has also been found to have a protective pharmaco-dynamic effect on a myopathy in sheep known as "white muscle disease" (194).

In a recent study, the administration of Ca EDTA increased tolerance of rats to selenium injected intramuscularly as sodium selenite. The investigators Sivjakov & Braun (195), were able to increase markedly the number of rats surviving a dose of 10 mg per kg (LD_{50} is 7.5 mg per kg), if the drug is given within five minutes after the selenite was injected. A similar type of alleviation of effects of selenium poisoning had been demonstrated earlier by DuBois (196) using 5 to 10 p.p.m. sodium arsenite or sodium arsenate in the diet.

OTHER TOXIC AGENTS

Since the toxicology and pharmacology of fluorides, radium, uranium, strontium, plutonium, rare earths, and beryllium were reviewed in last year's volume by Chen, Terepka, & Hodge (197), they will not be discussed here.

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