TOXICOLOGY: INORGANIC[1]

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Any attempt to review the field of toxicology and to extract the ripest kernels of information from the flood of current publications of even the past few years will yield unsatisfactory results for a fairly large proportion of the investigators and practitioners who turn to such a review as a means of acquainting themselves with the present state of this science and art. If this statement is regarded as evidence of the dissatisfaction of the authors with the present product, it may properly be accepted as such. But the issue goes much deeper than this personal attitude, and relates to the present complexity of the field, and to the need of the toxicologist to cope ever more effectively with the practical problems of a society that is confronted by the burgeoning technology of our time.

Classical toxicology, without suffering absolute loss in the quality of its professional challenge and social significance, has been displaced from the focal point of scientific concern by industrial and environmental toxicology, and this development has altered the present and ultimate scope of toxicology, as well as the primary aspect (the superficial appearance) with which it now faces the practical world. The widening of the scope of environmental toxicology to embrace virtually the entire field of general and specialized physiology is the source both of satisfaction and dismay to scholarly investigators (and teachers) in the field, who recognize and respond to the increasing richness of the intellectual stimulus, but who realize also the demand for haste in contributing to the armamentarium of preventive medicine and hygiene. The urgency of the latter, as it relates to the general public (as differentiated from the workmen in industry), may not be as great as the volume and intensity of the spoken and written word on the subject would indicate, but it has led to an impressive multiplication of the personnel and conventional methodology of environmental toxicology, and to a voluminous output of work and scientific publication in this field, without a proportional degree of clarification of the essential character of the problems, or a corresponding development of physiological insight into the meaning of the accumulating data. Under such circumstances, any reviewer, laboring under the restrictions of available time, with his own limited experience and viewpoint, serves but a segment of his scientific colleagues, and acknowledges an unfulfilled obligation to many others.

Carbon monoxide.—Two interesting aspects of the clinical syndrome of carbon monoxide poisoning have been reported recently. It is generally believed that the conversion of 70 percent of the hemoglobin to carboxy-

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hemoglobin is required to produce death, but Dalgaard (1) reports that the conversion of 40 percent of the hemoglobin to carboxyhemoglobin may be lethal for elderly persons. He also states that the "cherry-red" of carbon monoxide poisoning is evident unless the concentration of carboxyhemoglobin is below 40 percent. The electrocardiographic changes in carbon monoxide poisoning have been reviewed by Cosby & Bergeron (2), and by Middleton, Ashby & Clark (3), and they are agreed concerning these effects, although the latter group also describes conduction defects. The findings may be due to anoxia, specific toxic effects, or, possibly, secondary shock.

The search for evidence of the occurrence of chronic carbon monoxide intoxication, as represented by prolonged exposure to low concentrations, continues. Lindgren (4) reviewed the literature of carbon monoxide poisoning and found that there is an increase in the incidence of headache among persons whose exposure results in concentrations of carboxyhemoglobin of the order of 7.5 percent. A group of workmen exposed to concentrations of carbon monoxide ranging from 10 to 150 ppm (average 50 ppm) were studied by Hofreuter, Catcott & Xintaras (5), and, at this level, the average concentration of carboxyhemoglobin in the blood of the exposed group differed significantly from that of a group of persons not so exposed. Porphyrins were found by Shul'ga (6) to be depressed in persons who were working in an atmosphere containing carbon monoxide in the concentration of 27 parts per million. When the yeasts, *Saccharomyces italicus* Castelli, were cultivated in an atmosphere containing 2 percent of carbon monoxide, the nucleic acids were increased [DNA was increased 18 to 19 times, RNA was increased 33 percent (7)]. Although it is generally agreed that the deleterious effect of the inhalation of carbon monoxide is due to anoxia, it is usually mentioned that it also blocks the action of cytochrome oxidase, but Joels & Neil (8) remind us that the affinity of cytochrome oxidase for oxygen is six times that for carbon monoxide, and the K value for myoglobin is only 77, as compared to 210 for hemoglobin.

The relative merits of oxygen versus carbogen in the treatment of carbon monoxide poisoning is reviewed by Ledingham et al. (9, 10). The combination of 5 percent of CO_2 and 95 percent of O_2 is most effective in lowering the concentration of carbon monoxide (carboxyhemoglobin) in the blood. These authors describe the theoretical and practical value of oxygen under increased tension in the therapy of carbon monoxide poisoning. The theoretical value of hypothermia has again been advanced by Lorhan & Brookler (11), and should be considered in the treatment of severe poisoning.

Oxides of nitrogen.—Cases of "Silo-filler's disease" continue to be reported, and a review from the Department of National Health and Welfare (12) in Ottawa states that concentrations of nitrogen dioxide ranging from 200 to 4000 ppm have been found in silos. From the observations that have been made and reported, it would appear that a single respiratory exposure to the oxides of nitrogen in sufficient concentration in the air under these conditions is capable of inducing progressive fibrosing bronchiolitis (13). The pulmonary edema, which is an early effect, is followed later by the

onset of bronchiolitis, which may or may not be reversible. The frequency with which this syndrome occurs and the spectrum of the severity of the bronchiolar lesion are not known.

The relationship of chronic pulmonary disease to prolonged exposure to nitrogen dioxide and other pulmonary irritants is unsettled. Gough (14) believes that centrilobular emphysema is associated with bronchiolitis, as well as with bronchitis. When Carson et al. (15) subjected animals to air containing nitrogen dioxide over short periods of time, there was evidence of ocular and respiratory irritation. At levels which produced pulmonary edema in the rat, the dog showed only mild signs of pulmonary irritation. When animals were subjected to the inhalation of air containing this gas in the concentration of 25 ppm, by Wagner and associates (16), no pathologic lesions were found in any species. Rabbits sustained a transitory decrease in their uptake of oxygen. The lesion of bronchiolitis obliterans has not been produced in experimental animals, and it remains to be determined whether this is limited to the human species. Chronic pulmonary disease, comparable to human emphysema, has not been produced in animals by prolonged exposure to pulmonary irritants, although Boren (17), using a combination of nitrogen dioxide and carbon particles, and Freeman & Hayden (18), using low concentrations of nitrogen dioxide, have found destructive changes in the lungs of rats.

An interesting protective mechanism, and possibly a factor in susceptibility, was described by Pace et al. (19). In experiments with tissue cultures, they found that cells in a medium of serum survived in an atmosphere containing nitrogen dioxide in the concentration of 2400 ppm, whereas a concentration of 100 ppm under otherwise comparable conditions, was lethal when the culture medium was saline solution.

Ozone.—Ozone has acquired increasing toxicologic importance recently, because of its apparent role in air pollution. It is recognized as a respiratory irritant in industry, in which its threshold limit in the air breathed regularly by workmen was recommended in 1962 as 0.1 part per million. Even in the far north, where there is little or no air pollution, ozone is being formed continuously in the atmosphere, from which it disappears within 60 min when the sun is obscured, according to McKee (20).

The problem of the additive effects of several irritant gases in the atmosphere of modern urban centers is important, both theoretically and practically. Svirbely, Dobrogorski & Stokinger (21) have found that low concentrations of hydrogen peroxide in the atmosphere which rats were compelled to breathe enhanced the toxic effects of usually noninjurious concentrations of ozone, and, conversely, that repetitive exposure to a low concentration of hydrogen peroxide provided a moderate degree of protection to rats against otherwise lethal concentrations of ozone. Such acquired protection or tolerance was found by King (22) to persist for more than two weeks. Stokinger & Scheel (23) also found that this type of tolerance was imparted by exposure to other edema-producing agents (i.e. NO_2). Protection was afforded against acute effects only, however, and not against bronchitis or bronchiolitis.

These investigators also found circulating antibodies in exposed rabbits, but they concluded that these had no relationship to the developed tolerance.

It is often stated that exposure to low concentrations of pulmonary irritants may be expected to increase the likelihood of pulmonary infection, and Purvis, Miller & Ehrlich (24) found mice to be more susceptible to infection following respiratory exposure to a low concentration of ozone. They postulated that the irritant action of ozone increased the secretion of mucus and that this delayed the clearing of bacteria from the respiratory tree. (It might also be inferred that this secretion would increase the clearance.)

The mechanism of action of the pulmonary irritants within the respiratory tree is not understood, but Fairchild & Graham (25) believe that oxidant gases, such as ozone, inhibit the action of the sulfhydryl system of the lungs, and that such inhibition is influential in the production of pulmonary edema. The observations of Skillen et al. (26, 27) suggest that the action of ozone is that of a pulmonary irritant, in that their exposure of rats to ozone resulted in an increase in the concentration of 5-hydroxytryptamine and a decrease in the monamine oxidase activity in the lung, while the concentration of 5-hydroxytryptamine in the brain decreased significantly. Fetner (28) found that cell cultures exposed to low concentrations of ozone showed multiple chromosomal changes.

Sulfur dioxide.—The obvious irritant effects of sulfur dioxide within the respiratory tree are well known, and the most commonly accepted threshold limit of the concentration in the air of industrial establishments in the United States, under the usual conditions of employment, is 5 parts per million. An investigation of the more subtle effects of the inhalation of sulfur dioxide upon the respiratory mechanics of healthy male subjects was carried out by Frank and colleagues (29), who found that there was an increase in the resistance to the flow of air in the respiratory tree following exposure to concentrations of sulfur dioxide as low as 1 ppm. This effect was noted within one minute after the initiation of the exposure, and it increased for about ten minutes, after which no further changes were observed. Salem & Aviado (30) studied the effects of the exposure of dogs to air containing this gas in the concentration of 200 ppm. They observed an initial bronchodilatation, and then bronchoconstriction, followed by a series of events that led to systemic shock. They suggest that certain reflexes, initiated by the inhalation of the gas, tend to restrict the severity of the exposure. Thus stimulation of the upper respiratory tract leads to apnea, and stimulation of the lower respiratory tract produces an expiratory blast that acts to clear the air passages of the dissolved gas.

In studying the acute effects of the inhalation of sulfur dioxide, Dalhamn & Strandberg (31) exposed the trachea of rabbits, *in vitro* and *in vivo*, to air containing sulfur dioxide in a concentration of 100 ppm and found that 90 to 95 percent of the gas under these conditions was absorbed in the upper respiratory tract. They concluded that, at the levels of sulfur dioxide commonly found in the ambient atmosphere of American cities, there would be no effect on the ciliary action in the respiratory tract. The physiological

mechanisms involved in the injurious action of sulfur dioxide within the respiratory tree, and specifically in the lungs, are not fully understood. In examining the effects upon cells, Thompson & Pace (32) exposed tissue cultures to sulfur dioxide in the concentration of 2000 ppm, and found that the medium bathing the cells dilutes and reacts with the gas, thereby protecting the cell culture. Their experiments demonstrated that the HeLa (human) is more susceptible to the effects of sulfur dioxide than is the mouse cell.

The Russian literature emphasizes the importance of sensory reactions as physiological bases for establishing environmental (atmospheric) thresholds, and Ryazanov (33) reports the induction of aberrations in certain conditioned reflexes of animals by the inhalation of air containing sulfur dioxide in the concentration of 0.23 ppm. Amdur (34) in 1961 expressed the opinion that the threshold for sulfur dioxide in the ambient atmosphere might reasonably be held to be of the order of 0.05 part per million. In an epidemiologic investigation in Nashville, Tennessee, it was found by Zeidberg, Prindle & Landau (35) that asthmatic persons in the population had more frequent attacks when the concentration of sulfur dioxide was elevated in certain urban areas, but this was not found to be true of the entire population under investigation.

Finely divided, solid particles.—Particulate, inorganic compounds and mixtures of compounds, dispersed in the ambient atmosphere of communities as a whole, or of various parts of communities (e.g., along highways in specific commercial and industrial areas), and within industrial establishments, provide an increasing series of problems relating to environmental health. Not only are many of these particulate materials harmful, in themselves, under appropriate conditions of form and dosage, but even the most innocent of them may be the means of conveying other more harmful gases or aerosols adsorbed upon their surface or otherwise bound to them, into the respiratory tree, and thence into other parts of the body.

Extensive investigations are being made, that may be expected to multiply, in efforts to clarify the role played by the physical, chemical and biological characteristics of these particulate materials in relation to their immediate, intermediate and ultimate fate within the respiratory system and the body of the organism, as well as the effects exerted upon the organism. An adequate review of recent contributions to this general subject would go far beyond considerations of present practical consequence and would greatly exceed the scope of this review. Only one of the many substances in this category will be dealt with briefly.

Silica.—The mechanism of the production of the severe, disabling disease of man produced by silica continues to interest chemists, physiologists, and immunologists. Silica, as opposed to other dusts, produces an intense and specific fibrotic reaction, and it is this unusual fibrosis that intrigues these scientists. Theories to explain the production of this lesion have been based on the mechanical, physicochemical (including solubility and surface action) and biological characteristics of silica in the media of the tissues of the body. The most recent reviews regarding pathogenesis have been those of Vigliani

& Pernis (36), and of Policard (37). Both of these papers emphasize, convincingly, the biologic response of the organism to silica.

There is little difficulty in recognizing the intense, whorled fibrosis in the pathologic lesion of silicosis, and Strukov & Raikhlin (38), recently, have reported histochemical comparison of the reactions of silicosis and anthracosis. They found more necrosis in silicosis, in which also the reaction is more cellular and more rapidly proliferative. Collagenase is more active in removing procollagen, and there are more mucopolysaccharides. Heppleston (39) described an interesting experiment which helps to explain the progressive fibrosis which is often seen clinically. When he exposed silicotic rats to air containing finely divided coal or hematite, he found that these dusts entered the fibrotic nodule and also moved into the hila of the lungs. This evidence would suggest that the nodules are not static, and that alveolar fibrosis occurs before the draining lymphatics are blocked.

Despite the definite fibrogenic property of silica, it is believed by most clinicians and investigators that not all those exposed to the dust will develop disabling silicosis. The investigation of Vorwald (40), in which he quantitated the mineral content of lungs, emphasizes this problem, in that there was distinct overlapping in the amounts of free and total silica in non-exposed lungs, exposed normal lungs, and silicotic lungs, although the silicotic lung generally contained higher concentrations of silica. The lung has an efficient mechanism for clearing itself of inhaled foreign matter, such as quartz, but the interesting study of Gross (41) showed that tuberculin-positive guinea pigs, when exposed to dead tubercle bacilli and quartz, suffered an intense reaction, with subsequent pulmonary edema which washed out the dust from the alveoli.

In an attempt to explain the action of quartz, Marasas & Harington (42) investigated the oxidant activity of dust *in vitro* and found a positive correlation between the fibrogenic properties and the oxidant activities of various dusts. Parsons & Denstedt (43) approached the problem biochemically and found that the peritoneal response is intense inflammation, followed by proteolysis, when quartz is injected into the peritoneal cavity. This is followed in a few days by the action of antiproteases which terminates the proteolytic process. The well known, abnormal patterns of the serum protein (44) and the content of globulin in the silicotic nodule have evoked much interest. Many immunologic aspects of silicosis are intriguing. MacNab & Webster (45) have found that silica lowers the serum properdin sharply to the extent of 50 percent in rabbits, although the level returns to normal in about three months. Pernis & Paronetto (46) have found that silica-treated animals produce more antibodies than untreated animals. Using the techniques of fluorescence, Jones & Heppleston (47) could find no antiglobulin in the silicotic nodule similar to that which was found in the serum.

The relationship of Caplan's syndrome to simple silicosis and to rheumatoid arthritis directs attention to the role of protein in silicosis. Payne (48) found no differences in the serum protein patterns in these diseases. When Schroeder, Franklin & McEwen (49) examined the rheumatoid factor in

silicotics without rheumatoid arthritis, they found it similar in all measured respects to that of rheumatoid arthritis. Gorringe (50) believes the Caplan's lesion is the site of the production of the rheumatoid factor.

The Committee on Occupational Diseases of the Chest, American College of Chest Physicians (51), recently recommended the inhalation of powdered aluminum in the treatment of silicosis. This decision was based on experimental evidence indicative of its value. However, a recent report from Ontario (52) revealed no definitely beneficial effects from the prolonged use of aluminum in such therapy. In addition, Stoeckle and associates (53) have described a case of pulmonary fibrosis which appeared to have been produced by prolonged exposure to aluminum powder. Moreover, in the same group of workmen (exposed similarly to aluminum powder) in which this case was found, X-ray films of the chest disclosed a greater number of abnormalities than were expected under the occupational conditions which prevailed. On the basis of present knowledge, it would hardly seem that these effects can be attributed to aluminum, but it is also apparent that the intensity of the respiratory exposure to aluminum is greater, as a rule, when it is employed therapeutically than it is under occupational conditions. Experimentally, Talley & Burrows (54) found that hydrocortisone partially blocked the progression of the silicotic lesion, but when this treatment was discontinued, the further progression of the lesion was uninhibited.

Paul (55) describes the natural history of the silicotic lesion in man, and his data again implicate tuberculosis. It is often stated that chemotherapy is frequently ineffectual, but the paper of Morrow (56) describes good results with isoniazid therapy.

Metallic and Metalloid Poisons

The materials dealt with above have been classified as contaminants of the air for the reason that they gain entrance into the human body, for all practical purposes, only in that medium and by way of the respiratory tract. Those which are listed below in a different category have additional avenues of entrance and absorption and hence offer a more complex series of toxicologic and hygienic problems for solution. The metals, as a group, are widely distributed in nature, occur naturally or as the result of human artifice, in varied quantities and in varied patterns of distribution in the human body, and exert poisonous effects at various sites, in accordance with their physical characteristics and chemical reactivity in interposing interferences into essential cellular processes. With all due regard for both the brilliant and the pedestrian contributions which have been made to the interpretation of the action of specific members of the group, the basic mechanisms of their behavior are still shrouded in mystery, so that the avid investigator examines each new experimental report minutely for a new means of penetrating this obscurity. It is in this spirit that the results of a few of those recent investigations which appear to have the greatest impact, at present, on human health, have been assembled. To have explored this entire field with the minute care and perspicacity that would be required to find all of the signifi-

cant contributions, or even those which will be found, ultimately, to be the most important, would have exacted much more of time and erudition than was available. That which has been found and appraised has appeared to be of value to both the investigator and the practitioner.

Arsenic.—The syndrome of acute arsenic poisoning is well documented, but two interesting clinical highlights are worthy of mention. A diagnosis in acute arsenic poisoning (57) was made after radio-opaque material was found by X ray in the region of the stomach and pancreas, and then the lavaged material from the stomach was analyzed. Weinberg (57a) reported electrocardiographic changes in acute arsenic poisoning which were characterized by striking evolution of the T-wave.

Hanna & McHugo (58) reported a physiologic investigation of the effects of inorganic arsenic on small mammals. They described in detail the responses which are characterized initially by an immediate dilatation of capillaries, with a subsequent fall in blood pressure and a loss of plasma proteins.

Arsenic has long been implicated as a carcinogenic agent. One of the sites at which its action is believed to have been exerted is the skin, and Graham, Mazzanti & Helwig (59) have studied Bowen's disease, a chronic precancerous lesion of the skin. On analyzing the tissue from Bowen's disease they found an abnormally high level of arsenic, as compared to analogous tissue from normal persons.

On determining the content of arsenic in the lungs of patients, Holland, Acevedo & Clark (60) found the concentration of arsenic in cancerous lungs to be twice as high as that in the lungs of controls. In none of these cases was there known occupational exposure to arsenic.

To further the understanding of the biochemical action of arsenic, Wadkins (61) studied the effects of arsenate in the mitochondria of the liver of rats, and found that there was uncoupling of oxidative phosphorylation. This reaction was subject to reversal by phosphate ion. He states that arsenate stimulates ATPase activity. Fletcher, Fluharty & Sanadi (62), working with this same biologic system, found that arsenate did not bind the essential dithiol grouping unless BAL was present.

Cadmium.—As a part of a survey of the trace metals, Schroeder & Balassa (63) reviewed the metabolism of cadmium. They found that there is virtually no cadmium in the newborn, and then gradually there is an increase in the concentration in the tissues with age. The highest levels were found in the kidneys, and next highest, in the liver. Cadmium is apparently a contaminant in man; however, in the horse, cadmium has been found to be a normal constituent of the cortex of the kidney.

The inhalation of cadmium as a fume, in sufficient concentration, produces pulmonary irritation and pneumonia. Under the conditions of more prolonged occupational exposure to finely divided compounds of cadmium, proteinuria may result, and emphysema has also been reported among persons so exposed. Smith, Smith & McCall (64) reported five cases of emphysema. There had been no associated bronchitis in these cases, and al-

though there were no specific or characteristic features in their course, the authors thought that the occurrence of five cases in an occupational group suggested a causal relationship.

The proteinuria has been characterized by Smith, Wells & Kench (65) and by Piscator (66). They find a preponderance of globulins, especially alpha-2, and the proteins are rich in carbohydrates. These findings would suggest that the lesion is tubular, since the proteins in the urine are not similar, in their pattern of occurrence, to those in the serum.

In seeking to elucidate the cause of the anemia of cadmium poisoning, Berlin & Friberg (67) found that chronically poisoned rabbits had a normal myelopoiesis in the bone marrow. Berlin, Fredericsson & Linge (68) found the anemia to be due to increased destruction of the red blood cells.

Various experiments have revealed the following facts concerning the handling of cadmium by the body. A study of the metabolism of cadmium in the mouse, using cadmium 69, revealed that there is a marked retention, primarily in the nonosseous tissues, although Worker & Migicovsky (69) found that vitamin D increases the deposition of cadmium in the skeleton. Perkins (70) states that cadmium combines with human albumin at the same sites as zinc, and it reacts generally in a manner similar to that of zinc. It is also found to inactivate alkaline phosphatase in *Escherichia coli*, and it is thought that this is the result of the replacement of zinc in the enzyme.

In an investigation of cadmium as a potential carcinogenic agent, Heath et al. (71) injected pulverized metallic cadmium into rats, whereupon tumors developed in 15 of the 20 experimental animals. The main structure of the tumors was that of rhabdomyosarcoma.

Chromium.—In a systematic approach to trace metals, Schroeder, Balassa & Tipton (72) reviewed the metabolism of chromium. They found that the concentration of chromium in most of the tissues of infants is relatively high, and is subject to decrease during the first two decades of life. There is little tendency toward the accumulation of chromium in the body, but small quantities are almost invariably found in the lung. Despite the presence of chromium in the lungs, and its apparent association with pulmonary cancer among certain groups of men whose work involves respiratory exposure to chromate, these reviewers found no evidence on which to link these facts with cancer of the lung in the general population. The metal and its compounds are poorly absorbed, perhaps as the result of a natural barrier, and minute quantities are found regularly in the urine.

In understanding the toxicology of any element, it is important to know if it is an essential nutrient. Recently, Mertz, Roginski & Schwarz (73) have found that chromium is active normally in the removal of glucose from the blood and that it enhances the uptake of glucose by adipose tissue. In the liver of rats, Schroeder, Vinton & Balassa (74), found that chromium enhances the synthesis of cholesterol and fatty acids.

Hexavalent chromium is important as an occupational allergen. Anderson (75) has found that the hexavalent ion is tightly and, perhaps, permanently bound to the skin, under the conditions that prevail normally in the

tissues. By using a reducing agent, sodium pyrosulfite, and a chelating agent, tartaric acid, Samitz, Gross & Katz (76) were able to remove the chromium ion from the skin.

Collins, Fromm & Collings (77) found that the renal clearance of chromium by the dog is exponential. They stated that tubular excretion is probably minimal, and that most of the chromium is excreted in the urine as the trivalent ion.

Many attempts have been made unsuccessfully to produce cancer in experimental animals with chromate, but recently Payne (78) was able to produce tumors in mice with the residue of roasted chromate ore.

Iron.—Cases of acute poisoning, following the ingestion of comparatively large amounts of various compounds of iron, continue to appear. Arena (79) has pointed out an interesting feature of iron poisoning, in that there is no organic mechanism for the excretion of an excess of iron. Block (80), in his review of the pharmacology of iron, states that man loses 1 mg of iron per day through the combined routes of the bile, urine and sweat, together with that involved in the exfoliation of cells. The pattern of fatal cases of iron poisoning includes restlessness, hematemesis, drowsiness, collapse, cyanosis and coma. When recovery occurs, there is frequently residual scarring in the stomach, especially at the pyloris, as well as fibrosis of the liver. It is generally believed that the intestine acts as a barrier to the absorption of iron, and Schafir (81) has reviewed the possible mechanisms by which an overload can occur. Despite the lack of evidence as to whether the iron enters through a storage barrier, or whether such barrier is overloaded, or actually exists, there is no doubt that iron in toxic concentrations is capable of gaining access to the blood stream via the intestine. (The concept of the existence of a "barrier" to the absorption of a material into or through a specific tissue or membrane, or surface, would seem to be acceptable only as a somewhat naive, albeit expressive, substitute for mechanistic information.)

Because of the toxic manifestations associated with the use of iron compounds in oral therapy, a parenteral route of administration is often used. In investigating the effects of the parenteral administration of iron, Goldberg, Martin & Smith (82) found experimentally that when iron is injected into rats, a large proportion of the dose remains at the site of the injection, but in other species, most of the iron is carried to the liver. Witzleben & Chaffey (83) found that the iron in the liver was contained in the reticuloendothelial cells for a short time (approximately 1 week), but by the end of five months, the residue was found in the hepatic cells. This iron in the liver is not inert, but apparently binds sulfhydryl groups and, in addition, inhibits glucose-6-phosphatase.

Concerning the carcinogenic properties of iron, Lundin (84) holds that the injection of iron compounds of low molecular weight does not produce tumors. He believes that iron is carcinogenic under appropriate conditions, but that the carrier must be of sufficient molecular weight to produce a histiocytic reaction. When iron is injected parenterally, there is a high concentration of iron locally, and Fielding (85) states that the tissue response

includes reticulo-endothelial stimulation, increased production of tissue peroxides, and impairment of the metabolism of vitamin E. The local lesion is initially histiocytic and later fibroblastic. In directing further attention to the carcinogenic properties of iron, this author cites the association of hepatoma with the disease of iron storage hemachromatosis, and the occurrence of cancer of the bronchus among men who work with hematite. It would appear that the sarcoma produced in rodents by the subcutaneous injection of iron compounds is related to the relatively large amount of iron that persists at the site of injection; and corresponding conditions, with respect to the concentration of iron as well as the induction of tumors, do not occur at other sites.

Calcium EDTA has been found to be effective in removing iron from the tissue of experimental animals. Barrie & Wilson (86) have also reported a strikingly effective excretory response to such therapy, as represented by the large amounts of iron in the urine of a patient poisoned by iron sulfate. The amount excreted per day exceeded the pretherapeutic level approximately one hundred fold.

Lead.—The metabolism of lead in man has been reviewed recently by Schroeder et al. (87), and the experimental work of the Kettering Laboratory has been summarized by Kehoe (88). They discuss the patterns of ingestion and excretion and Kehoe states that an approximate equilibrium is established normally, at an early age, between the intake and output of lead of persons in the United States. Kehoe argues that, because of the multitude of opportunities for the contamination of food and beverages with lead, every reasonable effort should be made to limit such contamination through the careful handling and processing of food and beverages, including community supplies of water (the latter to not more than 0.05 mg per liter, food to not more than 0.6 mg per average day per adult). In determining the lead content of the tissues of an impressive number of apparently normal males, Tipton and Cook (89) found the median concentration of lead in the heart to be 10 micrograms per gram of ash, in the kidney, 98 μg per gram of ash, and in the lung, 49 μg per gram of ash. Black (90) investigated the behavior of lead 210 in dogs. As would be expected from other data, the rate of the urinary excretion of lead did not correlate with the body burden of lead. Under the conditions of his experiments, 90 percent of the total body lead was in the skeleton. He estimated that 5 percent of the lead in the urine was derived from the skeletal tissues, and that this may have represented 0.05 percent of that in the entire skeleton.

From the clinical viewpoint, several items of interest have been reported. Pease & Newton (91) have reported that metaphyseal dysplasia occurs in 50 percent of children who have had lead poisoning. This lesion also occurs in the child in the absence of symptoms and is represented, in pathologic terms, by a zone of compactness at the metaphysis, adjacent to which is a zone of packed trabeculae of calcified and plumbified cartilagenous matrix.

Several matters of interest relate to the kidney. Radošević et al. (92) reviewed 53 cases of clinical lead poisoning, and found that although only 3

had abnormalities in the urine, as disclosed by the usual type of urinalysis, 21 of these cases had elevation of the BUN, or impairment of concentrating ability, phenol red excretion, or urea clearance. Another interesting aspect of renal injury in lead poisoning concerns the occurrence of the triad of the Fanconi syndrome. Chisolm & Leahy (93) report that this occurs in the severely intoxicated, but is reversible. These findings suggest a specific site of tubular damage. Inclusion bodies have been noted in renal tubular cells for many years, and they were described again in a clinical case of lead poisoning (94). The nature of this lesion is described in Watrach & Vatter's book *Electron Microscopy* (95). The essential units of the inclusion bodies are fibrils which are densely packed, interspersed with numerous ferritin granules. The inclusion bodies contain a small amount of DNA and a large amount of protein with a high content of SH groups. Beaver (96) states that these intranuclear inclusions differ from those of viral origin. In the rat, they are in the proximal convoluted tubule. He could not demonstrate lead in the inclusions, however. Interestingly, he states there was no tubular dysfunction in the rats.

Another aspect of renal pathology in lead poisoning concerns the occurrence of renal tumors in experimental animals. Dukes (97, 98) fed a diet containing 1 percent of lead acetate to rats, and 15 of 16 developed tumors. He questioned whether the porphyrin in the urine may have been the causative agent, but he could not produce tumors with a different porphyrin. Van Esch, Van Genderen & Vink (99), investigating the renal tumor of rats induced by lead acetate, found that these animals developed cystic nephritis, and in the walls of these cysts, papillomatous outgrowths were noted.

The ability of calcium EDTA to enhance the urinary excretion of lead is generally accepted, but its value, since the body burden may be in excess of 1 gram (100), is questionable. In addition, although amino-aciduria and glycosuria are reported in lead poisoning, Andrews (101) reported several cases in which these constituents of the urine appeared only after therapy with EDTA. The mechanism which is responsible for this effect is not clear, but Andrews postulates that the EDTA may chelate a trace metal in the kidney. The use of penicillamine as a chelating agent, instead of EDTA, has been suggested because of the toxicity of EDTA. Ohlsson (102) reported that penicillamine induced an increase in the output of lead in the urine equal to that caused by EDTA, but Mosser & Bessman (103) found EDTA more effective. Meltzer, Kitchell & Palmon (104) reviewed the toxicity of EDTA, and report that although there is a potential danger of nephrotoxicity, 50 mg per kg per day is a safe therapeutic level.

Because of increasing concern as to the effects of pollution of the ambient air of urban centers with lead, an interest in the establishment of a threshold level compatible with the safety of all segments of the general population has grown. The balance experiments of Kehoe (88) and his associates have demonstrated that when the respirable lead in the atmosphere of a normal adult, on a diet of normal lead content, does not exceed 0.15 mg per m^3, during

approximately 40 hours of exposure per week, the absorption of lead will remain within safe limits indefinitely. It is not possible, however, on the basis of the available information to make a satisfactory estimate of a correspondingly safe concentration for conditions of exposure that would be continuous. Russian investigators following a different experimental philosophy and design, have expressed highly divergent views with respect to both occupational and general environmental exposure to air-borne lead. Gusev (105) has reported that there is no disturbance of the higher nervous centers associated with the conditioned reflex if the concentration of lead in the industrial environment is kept under .001–.004 mg per m^3. He and his compatriots believe that the maximum concentration of lead in the general ambient atmosphere should not exceed one-fourth of the industrial occupational threshold. As a further provision for the safety of workmen in lead-using industries, the Russian recommendation (106) includes a list of 17 major disease processes which should disqualify a man for such employment. It is interesting, in relation to professional and scientific (or other) attitudes, to reckon with the fact that the limit of the concentration of lead, so recommended as an appropriate requirement for the safety of workmen in the lead-using industries in Russia, is of the order of the concentration of lead in the ambient air of several representative cities in the United States (88).

Mercury.—The classical signs of poisoning induced by metallic mercury—psychic disturbance, tremor, and gastro-intestinal symptoms—are not always seen. Burke & Quagliana (107) reported a case of poisoning from the inhalation of mercury vapor that was characterized by pulmonary edema and acute necrotizing bronchiolitis. The concentration of mercury in the urine is an important diagnostic criterion for this poisoning, but because of the variability in the rate of the excretion of mercury in the urine from individual to individual and in the same individual from time to time, it is necessary to exercise great care in the interpretation of individual analytical findings, and, at times, the results of a more elaborate analytical investigation of a patient may be inconclusive. Usually, however, it is possible to secure analytical data that will differentiate between potentially significant and wholly insignificant levels of excretion. Evans (108) recently stated that symptoms will occur when the concentration of mercury is above 300 micrograms per liter of urine. Most experimental investigations of urinary excretion of mercury have been made following subcutaneous or intramuscular injection, but Gage (109) has studied the pattern of the excretion of mercury by rats which had been exposed to mercury vapor. When the air inhaled by the animals contained 1 mg of mercury (Hg) per cubic meter, approximately 50 percent of the mercury was absorbed from the respiratory tract, and only a minute proportion of the absorbed mercury was excreted into the gut. Rats subjected to the inhalation of air containing this concentration of mercury reached a steady state in 10 days. A rapid turnover of the mercury occurred in all tissues except the brain, the tissues, generally, being cleared of mercury in one week after the termination of the exposure. The pattern of excretion in the urine was not a simple

logarithmic curve, and there appeared to be a delaying mechanism in the kidney, whereby excretion might be expected to continue for upward of six months.

While the renal lesion in mercury poisoning is virtually limited to the proximal convoluted tubule, five cases of nephrotic syndrome were recently described which were thought to be the result of the absorption of mercury from ammoniated mercury (110). Rodin & Crowson (111) investigated the nephrotoxicity of mercury in the rat. With increasing dosages, the lesion in the proximal convoluted tubules extended proximally. They believe that the site (extent) of injury is determined by the concentration of mercury in the filtrate, and as the urine passes down the tubule it becomes increasingly concentrated.

The point of action of mercury ion in the proximal convoluted tubule that produces diuresis is the sulfhydryl group. Miller & Farah (112, 113) state that, if these groups are blocked, diuresis will be prevented. This will result when *p*-chloromercuric acid, rather than a simpler compound of mercury, is administered. Approximately 20 percent of the sulfhydryl groups in the proximal convoluted tubule are concerned with diuresis.

Several other interesting biological actions of mercury have been reported. Arbuthnott (114) found that mercury will produce hemolysis. Just as lead and copper, mercury enters into a chelation or coordination complex with the erythrocytes which causes these to clump. Porphyria (115) has been produced by several mercuric compounds.

Nickel.—A comprehensive review of nickel by Schroeder, Balassa & Tipton discussed the normal metabolism of this metal in man (116). This element is not known to be required in any enzyme system. Normally it is found in the kidney, liver and lung, and it accumulates in the lung with age. The only toxic manifestation other than that derived from "sensitization," was the apparent carcinogenic potential of nickel, as displayed in the respiratory tract of certain workmen in nickel refineries. (This apparent lack of toxicity on the part of the inorganic compounds of nickel would appear to be due to the slight, almost negligible, absorption of these compounds. Certain organometallic compounds that gain access to the tissues of the body are not so innocent.)

Nickel has been found to be an active sensitizer of the skin under conditions of repetitive or prolonged contact therewith among industrial employees, as well as those who have worn fabrics containing finely divided catalytic nickel. An interesting case of Löffler's syndrome was reported by Sunderman & Sunderman (117), following the exposure of a workman to nickel carbonyl. Stoddart (118) has described another case, which may have been a manifestation of the allergenic property of nickel, in which an anaphylactoid type of local reaction occurred following the insertion of a nickel-plated cannula into a vein.

The implication that nickel has carcinogenic characteristics is commonly accepted. The exposure of workmen to finely divided nickel in refineries has

been associated with a significant incidence of respiratory tumors. Gilman & Ruckerbauer (119) have injected a preparation of such finely divided nickel (from the source mentioned above) into rats and mice and have produced sarcomas in approximately 40 percent of the rats. In his further investigations, Gilman (120) found that nickel sulfide induced a higher incidence of tumors than did nickel oxide; nickel sulfate was inactive in this respect. Sunderman & Sunderman, Jr. (121) have found nickel in the tobacco of cigarettes, and postulated that the amount inhaled by a heavy smoker may be three times the carcinogenic dose for rats. (Inasmuch as the preponderance of certain of the mineral components of cigarette tobacco remain in the ash of the "smoked" cigarette, the validity or irrelevance of this postulate requires definitive demonstration.)

Selenium.—In a review of the toxicology of selenium, Cerwenka & Cooper (122) conclude that elemental selenium is relatively nontoxic, whereas hydrogen selenide is highly toxic (other compounds of selenium are represented in the review). From the experience of industrial personnel, it is known that selenium induces irritation of the respiratory, as well as the gastro-intestinal tract. The pathological lesions associated with intoxication are fatty degeneration of the liver, splenomegaly, gastro-enteric hemorrhage and mild tubular degeneration of the kidney.

Though it has been found recently that selenium is an essential element in the physiology of the rat, the chick and the lamb, it has not been thought to be essential to man (123). Schwarz (124) has described the first clinical application of selenium in the therapy of the nutritional disease, *kwashiorkor*. In Jamaica, patients with this disease failed to gain weight at a normal rate until they were treated with small doses of selenium daily (125). Vitamin E is closely associated in its function with selenium (123), but selenium will not prevent muscular dystrophy in the rabbit, nor will it prevent fetal absorption in rats suffering from deficiency of vitamin E. Olcott, Brown & Van der Veen (126) have pointed out that vitamin E functions as an antioxidant, and that certain seleno-amino acids have antioxidant activity. McConnell & Dallam (127) determined the distribution of selenium in the rat and found the element in the nuclei, mitochondria and microsomes, and in the supernatant fluid of the cytoplasma.

It is known that in acute poisoning, selenium occurs in some form in the exhaled air, and is responsible for an objectionable, garlic-like odor. Halverson, Guss & Olson (128) found that when sodium sulfate was administered to poisoned animals, the rate of the urinary excretion of selenium was augmented, without apparent alteration of the rate of excretion in the feces. Scott (129) offered the opinion that the mechanism of the toxic action of selenium is that of competition with sulfur for sites at which sulfur normally plays a role in cellular metabolism. Orstadius (130) found that the glutamic oxaloacetic transaminase in the plasma of the acutely poisoned pig was increased, but that the ornithine-carbamyl transferase remained within normal limits. It may be that these findings of Orstadius are characteristic of

the pig alone (or at least are not a general characteristic of selenium intoxication), in view of the striking and seemingly unique degeneration of the musculature of intoxicated pigs. In addition to the use of sulfates in the treatment of selenium poisoning, arsenic is said to counteract certain of its toxic effects, while brombenzene is said, by Ransone, Scott & Knoblock (131), to augment the excretion of selenium in the urine.

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