

### III. BIOLOGIC EFFECTS OF EXPOSURE

#### Extent of Exposure

Carbon disulfide, CS<sub>2</sub> (formula weight 76.14), is a colorless, volatile, and extremely flammable liquid at room temperature [1]. Its physical and chemical properties are described in Table XIII-1 [1-3]. The present method of manufacture involves the catalytic reaction of methane (natural gas) and sulfur vapor. Before about 1950, however, carbon disulfide was manufactured by the high-temperature reaction of charcoal with sulfur vapor [3].

In 1974, approximately 782 million pounds of carbon disulfide were produced in the United States [4]. As of 1971, approximately 53% of the carbon disulfide produced was used in the production of regenerated cellulose (viscose rayon and cellophane), and 25% was used in the manufacture of carbon tetrachloride. All other uses constituted the remaining 22% [5]. Some of these other uses occur in vulcanizing rubber (this use is becoming less common), in making rubber accelerators and neoprene cement, and in fumigating grain.

Because the viscose industry is the primary user of carbon disulfide and nearly all the studies included in this chapter involve workers in the viscose industry, the viscose process will be briefly described. The process begins with the steeping of sheets of pressed cellulose in a solution of sodium hydroxide to yield alkali cellulose. Next, the cellulose is shredded to make cellulose crumbs of consistent size. The crumbs are allowed to stand in air to depolymerize the cellulose crumbs. Carbon disulfide is then added to form sodium cellulose xanthate. This

xanthation process is accomplished in large churns, where modern machinery controls the exposure to carbon disulfide, although high-concentration exposures still occur occasionally. The viscose syrup is then filtered to remove undissolved particles. The filtered viscose is extruded through spinnerets into an acid bath to regenerate the cellulose. This "spinning" process converts the viscose syrup into filaments of regenerated cellulose or viscose rayon. The viscose rayon filament that is to become staple fiber is then cut into short pieces, washed, and dried. Carbon disulfide and hydrogen sulfide are evolved in a ratio estimated at from 2:1 to 10:1 [6(pp 5-8),7-10] during the spinning process and again during the cutting, washing, and drying. Hence, employee exposure is greatest in these areas.

NIOSH estimates that 20,000 employees are potentially exposed to carbon disulfide full-time in the United States; their occupations are listed in Table XIII-2 [11].

#### Historical Reports

Carbon disulfide was discovered accidentally in 1796 by the German chemist Lampadius, who observed it as the liquid product of a mixture of heated iron pyrites and charcoal [3]. Clement and Desomes, in 1802, obtained the compound by heating charcoal and elemental sulfur [3].

Carbon disulfide has since been used for a variety of purposes. In the 1840's, the Scottish surgeon Simpson tested carbon disulfide for its effectiveness as a narcotic-anesthetic. The compound was shown to have strong anesthetic properties, but its use was discontinued because it caused hallucinations, headache, and nausea in some patients and because its action was difficult to regulate [12]. Within the next decade, carbon

disulfide began to be widely used in industry because of its excellent solvent properties. It was used as a phosphorus solvent in the manufacture of matches and as a solvent in the preparation of fats, lacquers, and camphor, in the refining of paraffin, and in the extraction of oil from olives, palmstones, bones, and rags [13].

The first reports mentioning carbon disulfide as a potential health hazard came from France in the 1850's and referred to the India-rubber industry, in which carbon disulfide was extensively used [14]. At that time, factories in the modern sense did not exist; production took place in small, poorly-ventilated workshops, which were often a part of the craftsman's living quarters. In the manufacture of India-rubber, caoutchouc sap was softened with carbon disulfide, then spread out to produce rubber sheets. This process exposed the worker directly to carbon disulfide vapor [13].

A 1938 survey [13] reported 24 cases of carbon disulfide poisoning observed in 1856 by Dr. Auguste Delpech, who described the effects of this compound in this way:

He who works in the "sulphur" [CS<sub>2</sub>] is no longer a man. He may still make a living from day to day in unskilled labor. He will never be able to establish an independent position for himself. The depressing influence of the carbon disulfide upon his will power, ...the painful consequence of his indifference, ...the loss of his memory, prevent him from entering another occupation. Discouraged and haunted by self-contempt, these "miserables" are, moreover, robbed of those functions which human beings in all ages have held in highest esteem. Condemned to cruel isolation and deprived of loving care and affection at their own hearthstones--so often the only compensation and consolation of many an industrial drudger--these wretched creatures deserve, from the medical as well as from the social point of view, our deepest sympathy.

Delpech [15] described the case of the son of a rubber worker who, after 3 days of play in his father's workshop (and exposure to carbon disulfide vapor), was "stricken with a type of raging delirium" during which he "hurled himself at his father to bite him."

By the turn of the century, the rubber industry had expanded into large-scale production, and exposure was widespread. Severe occupational carbon disulfide intoxication in Europe continued to occur [16-18], despite the warnings of the early investigators. A 1902 British publication, Dangerous Trades [19], described a factory in which the windows of the vulcanizing room had to be barred to keep acutely poisoned men from leaping out during attacks of mania.

Foreman [20], in 1886, reported a rare case of carbon disulfide ingestion. A shoemaker, following a 10-day drinking spree, drank from a bottle of carbon disulfide (which he used in his work), mistaking it for gin. He died 2 hours later.

According to a 1938 survey of the viscose rayon industry [13], a German physician, Laudenheim, reported, in 1899, 50 cases of insanity that he attributed to carbon disulfide exposure, and stressed the importance of carbon disulfide as a poison of extrinsic origin capable of initiating distinct psychoses. Although it generated much controversy, this monograph was instrumental in alerting the European public to the risks of carbon disulfide in the rubber industry. In addition, Laudenheim was able to show that exposure to excessive concentrations of carbon disulfide in workplaces could be controlled at no excessive expense to the business. With ventilation improvements, carbon disulfide concentrations were reduced from several hundred to less than 30 ppm, with

a corresponding decrease in general and mental morbidity.

An 1892 paper by Peterson [21], a New York physician, was the first report of carbon disulfide intoxication in the United States. Peterson described three cases of insanity, which he attributed to acute carbon disulfide exposure, in employees of a rubber factory. These incidents had actually occurred 5 years earlier, but Peterson had delayed his report, thinking that he would hear of additional cases or acquire more information from plant owners or physicians. He was unable to do either, and he remarked on the secretiveness of the factory authorities regarding working conditions.

Bard [22], a California physician, reported two incidents of carbon disulfide intoxication, also in 1892. One involved the acute, nonoccupational exposure of two brothers who had purchased carbon disulfide for use as a rodenticide. A leaking 50-lb can of carbon disulfide was stored just above their bed, and the vapor would "descend to their faces" as they slept. This insidious exposure caused, within a few days, a transformation in the character of the brothers from "honest, industrious, and genial" to accusatory, suspicious, and paranoid. A bizarre sequence of events resulted in the suicide of one of the brothers. The other eventually recovered after a long period of mental derangement. The second incident occurred in the only plant producing carbon disulfide in California at that time. An employee imagined, without apparent cause, that a business associate was attempting to swindle him. The affected man fired two shots at his partner, for which he was charged with "assault to commit murder." He was later acquitted on the grounds that he was suffering from temporary mania due to inhalation of carbon disulfide vapor.

In the United States, the rubber industry was not so carefully regulated as it was in Europe, and scattered reports of intoxication resulting from exposure to high concentrations of carbon disulfide continued to appear through the first two decades of this century. In 1914, Hamilton [23] surveyed the incidence of industrial poisoning in the rubber industry and found that none of the plant physicians questioned was aware of the hazard of carbon disulfide, nor had they ever suspected it as being responsible for any form of illness. However, the foremen of the plants related a number of cases of intoxication that seemed to be caused by exposure to excessively high concentrations of carbon disulfide.

The introduction of the viscose rayon industry into the United States brought with it additional reports of carbon disulfide-induced intoxications [24,25]. It was not until a number of years later, however, that carbon disulfide gained notoriety in the United States as a significant occupational health hazard. Hamilton [14], in a 1925 review of the literature, mentioned two cases of intoxication that had been described to her personally by the attending physician. These early incidents in the viscose industry involved extremely high concentrations of carbon disulfide and presented a general picture of intoxication similar to those described in the rubber-works reports. Psychoses, tingling and numbness of the extremities, weakness of limbs, loss of appetite, weight loss, severe and localized headache, sexual dysfunction, impaired vision, and gastrointestinal disturbances were among the signs and symptoms reported following carbon disulfide intoxication [24,25].

Hamilton's 1925 report [14] does not seem to have been received with much concern. Twelve years later, in a presentation to the US Department

of Labor, Hamilton [26] remarked that, although the United States was at that time the second or third largest producer of viscose rayon, nothing had been done to alleviate the "deplorable condition" of worker health in this industry. A year later, in 1938, an extensive survey of the viscose industry was published by the Pennsylvania Department of Labor and Industry [13]. Following this, further reports appeared [27-29], and, in 1941, the first exposure standard was adopted by the American Standards Association [30].

#### Effects on Humans

Few recent reports or case studies have been found on acute effects of exposure to carbon disulfide.

Vigliani [31], in 1954, reported on his observations of occupational carbon disulfide poisoning in Italy. The first part of the study described 100 cases of carbon disulfide intoxication occurring during an outbreak of such cases in 1940 and 1941. Because of the war, the factories were operated at peak production, and employees were often exposed to carbon disulfide 10-12 hours/day at concentrations of up to 2.50 mg/liter (800 ppm), although the mean concentrations ranged from 0.45 to 1.0 mg/liter (144-321 ppm). In the 100 workers examined, polyneuritic symptoms were observed in 88% of the patients. Polyneuritis was diagnosed only in cases of absence or severe weakening of the Achilles or patellar reflexes. Usual symptoms included heavy, tired feelings in the legs, painful knees, and difficulty in walking. Gastric disturbances, headaches, and vertigo followed in prevalence with 28%, 18%, and 18%, respectively. "Sexual weakness" and tremors both occurred in 16% of the cases and myopathy in

15%. Psychoses were diagnosed in 5% of the 100 patients.

Vigliani [31] also reported on 43 viscose rayon workers with carbon disulfide poisoning, 39 of them from 2 viscose plants, who had been diagnosed as having encephalopathy between 1944 and 1953. The mean age of the affected workers was 52.8 years, with a mean length of exposure of 21 years. Mean concentrations of carbon disulfide found in the factories, as measured in 1943, ranged from 0.03 to 1.5 mg/liter (10-482 ppm). The first few cases observed were diagnosed as atherosclerotic dementia, pseudobulbar paralysis, diffuse encephalomyelitis, or cerebral thrombosis and were not considered occupational in origin. As these cases were observed more frequently, at ages younger than expected, and following long-term exposure to carbon disulfide, a relationship between chronic exposure to carbon disulfide and encephalopathy became apparent. Of the 35 affected workers under the age of 60, 16 had no hypertension, in contrast to the high probability of hypertension in presenile cerebral atherosclerosis. This indicated a possible toxic factor in the development of the encephalopathy. In some workers there was evidence of preexistence or coexistence of typical manifestations of intoxication by carbon disulfide such as polyneuritis. Vigliani described the typical course of the disease, based on the 43 observed cases. Asthenia, paresthesia, difficulty in walking, speech alterations, and mental deterioration were common early symptoms. Most workers had experienced a stroke followed by spastic hemiparesis. Extrapyramidal involvement occurred in 11 patients. Cerebral arteriography, EEG's, and examination of the fundus oculi indicated that the encephalopathy was vascular in origin. Necropsy of three patients revealed diffuse vascular sclerosis, cerebral atherosclerosis, hyaline



fibrosis of the media, and thickening of the intima of blood vessels. Most cases (84.6%) of vascular encephalopathy found in the two monitored plants occurred in workers from the spinning departments.

Lilis et al [32], in 1967, examined 26 viscose rayon workers using renal and hemodynamic tests. The workers, with a mean age of 46.6 years and mean length of exposure of 14.6 years, had been part of a previous study on cardiovascular effects of carbon disulfide exposure [33]. The investigators found that, at the time of examination, 7 workers were hypertensive, as diagnosed on the basis of blood pressure tests, 9 had a history of high blood pressure but were not hypertensive at time of testing, and 10 had never had high blood pressure. Effective renal plasma flow, total renal blood flow, systolic volumes, renal circulatory ratio, total renal resistance, creatinine clearance, and the filtration ratio (creatinine clearance/p-aminohippuric acid (PAH) clearance) were measured in the subjects.

Lilis et al [32] measured effective renal plasma flow by PAH clearance and found that 10 workers had normal PAH clearances (over 500 ml/minute), 9 had moderately low clearance (400-500 ml/minute), and the remaining 7 had greatly reduced PAH clearance (less than 400 ml/minute). The normal PAH clearance value of 580 ml/minute was determined as the mean value obtained from eight subjects with no history of exposure to carbon disulfide and no renal disorders, hypertension, or arteriosclerosis. The difference between the mean values of PAH clearance in the control group and the 26 exposed workers (580 versus 457 ml/minute) was statistically significant. Total renal blood flow levels were normal, ie, greater than 1,000 ml/minute, in only 10 of the 26 workers. The source of the "normal"

values, except as otherwise noted, was not given. Systolic volumes, measured in 22 of the exposed workers, were increased in 5 of 6 workers with marked reduction in PAH clearance, in 5 of 7 with moderately low PAH clearance, and in 3 of 9 with normal PAH clearance. Normal renal circulatory ratios (total renal blood flow/heart output) of 20-25% were found in 6 of 20 subjects; the other 14 had lower ratios. Normal total renal resistance (less than 70 dynes/sq  $\mu\text{m}$ ) was found in 7 of 23 workers; 10 had moderately increased resistance (70-100 dynes/sq  $\mu\text{m}$ ); and 6 had markedly increased resistance (greater than 100 dynes/sq  $\mu\text{m}$ ). Of the 12 workers with increased systolic volumes, 10 had increased total renal resistance. Three workers with marked reduction and two with moderate reduction of PAH clearance were tested for creatinine clearance. Four of the five had normal clearance and one had markedly reduced clearance. The filtration ratio (creatinine clearance/PAH clearance) was high or at the upper end of the normal range in four of five cases. The authors [32] concluded that long-term exposure of workers to carbon disulfide causes vascular disease, manifested in the alterations of renal function described above. These changes were suggested to be the results of activation of the sympathetic division of the autonomic nervous system, producing effects similar to those of epinephrine. The functional alterations were thought to evolve into organic renal lesions with longer exposure. The results of the renal tests appear to be reliable and valid, but the authors' explanation of the mechanism of the renal effects of carbon disulfide is not substantiated concretely by the data presented.

Ehrhardt [34] reported that a viscose rayon factory in the German Democratic Republic first employed women in 1950 but 6 months thereafter

prohibited their employment because of increased menstrual bleeding. However, at the time this report was published, in 1966, the author stated that the German Democratic Republic did not have a regulation prohibiting women from working where there might be exposure to carbon disulfide. Women were permitted to work where such exposures might occur if they were at least 20 years old, although women over 40 were preferred, and if the maximum allowable concentration (MAC) of 17 ppm (53 mg/cu m) was not exceeded. More recent surveys had not identified any instances of spontaneous abortions or complaints of amenorrhea, sterility, or general genital organ dysfunction. Ehrhardt concluded that, provided that the MAC is not exceeded and that women are carefully examined during employment, prohibition of all women from exposure to carbon disulfide is not necessary; pregnant women, however, should not be allowed to work where carbon disulfide is present. This study, a one-page review of recent experiences of the German Democratic Republic's viscose rayon industry with women in the workplace, cannot be considered as more than unsubstantiated qualitative conclusions.

Finkova et al [35], in 1973, reported on 35 women exposed to carbon disulfide in a viscose rayon factory, with emphasis on possible gynecologic abnormalities. A medical staff consisting of a neurologist, a psychiatrist, an ophthalmologist, and a gynecologist examined the women during 1967-1969. No significant neurologic, psychiatric, ophthalmic, or laboratory test abnormalities were observed. Gynecologic examinations, including inspection, palpation and cytologic smear testing, also did not reveal abnormalities in these exposed women. There was no evidence of irregular menstrual function, malformed fetuses, spontaneous abortions,

hormonal disorders, or altered sexual habits. Occupational disability records for all causes were examined for exposed and unexposed men and women in the plant. There were no discernable differences between exposed and unexposed workers nor between men and women. The authors concluded that carbon disulfide has no harmful influence on the health of women working in the viscose rayon industry. The report lacks in design, methodology, and quantitative data. No data on concentrations of carbon disulfide in the workplace were given, and results were merely described rather than statistically analyzed.

Jindrichova [36], as part of a study of 183 employees exposed to carbon disulfide at a mean concentration of approximately 200 mg/cu m (64 ppm) in a cord-fiber factory, reported that no specific reproductive system disorders were found in women workers.

The few experimental studies available on the effects of carbon disulfide exposure on humans have dealt primarily with skin absorption and metabolism.

Baranowska [37] conducted a study on humans to determine whether carbon disulfide was absorbed through the skin. Sixteen experiments were performed to test the absorption of carbon disulfide when the subjects' hands were immersed in aqueous solutions of carbon disulfide while pulmonary absorption of the vapor was prevented. The absorption of carbon disulfide vapor at unspecified concentrations through the entire surface of the body was also studied in two experiments. The quantity of carbon disulfide absorbed through the skin was estimated from the quantity of carbon disulfide exhaled. Baranowska assumed that the amount of carbon disulfide absorbed after a 1-hour exposure was twice the amount exhaled,

based on similar research of other investigators [38,39]. The effects of varying the concentration, temperature, and pH of the carbon disulfide solution were studied [37]. In experiments using carbon disulfide at increasing concentrations (0.35-1.67 g/liter) at a temperature of 21 C, the carbon disulfide absorption rate increased from 21 to 96  $\mu\text{g}/\text{sq cm}/\text{hour}$ . The effect of solution temperature on absorption was tested by performing similar experiments at a temperature of 40 C, using carbon disulfide concentrations of 0.2-0.8 g/liter. The 20-degree rise in temperature caused increased absorption. No significant differences in absorption rate were found when pH values were varied from 1.5 to 8.0. In the experiments testing dermal absorption of vapor, no carbon disulfide could be detected in the exhaled air. The author concluded that the dermal absorption rate of carbon disulfide from aqueous solutions was high enough to be of significant concern in the viscose rayon industry.

Mack et al [40], in 1974, studied inhibition of drug metabolism in 19 healthy men experimentally exposed to carbon disulfide. The men, 21-40 years old, were exposed for 6 consecutive hours in an inhalation chamber to carbon disulfide at 10, 20, 40, or 80 ppm (31, 62, 124, or 248  $\text{mg}/\text{cu m}$ ). Concentrations inside the chamber were monitored before and during each exposure. Groups of 4 fasting subjects were exposed at the various concentrations at intervals of at least 30 days. In the first of three experiments, each subject received 7 mg/kg of amidopyrine (AP) orally just prior to chamber exposure. The subjects were given two sandwiches after 2 hours of exposure and one sandwich after the 3rd hour. During the exposure they were allowed to drink a total of 1.25 liters of apple juice and mineral water. Urine samples collected 3, 6, 9, 12, 16, 24, and 33 hours

after the beginning of exposure were analyzed for metabolites of amidopyrine (AP), 4-Aminoantipyrine (AAP), and acetyl-4-aminoantipyrine (N-AcAAP). The excretion of the N-demethylation products was taken as a measure of alterations in microsomal enzyme activity. Preexposure urinary excretion patterns for AAP and N-AcAAP were determined, and the excretion averages for a 33-hour period were used to evaluate the changes produced by carbon disulfide. The 19 men served as their own controls by undergoing the experimental procedures without exposure to carbon disulfide. In the second experiment, 18 hours after a single 6-hour, 20-ppm (62 mg/cu m) exposure to carbon disulfide, a similar dose of amidopyrine was administered; the other procedures of the first experiment were followed. This experiment was designed to study the duration of inhibition of microsomal enzyme activity. In the third experiment, exposure to carbon disulfide at 20 ppm (62 mg/cu m), 6 hours/day for 5 consecutive days, with administration of amidopyrine at the beginning of the last exposure, was carried out to study possible cumulative inhibition.

The authors [40] found that, in the single 6-hour exposures, concentrations of 10 ppm (31 mg/cu m) caused no appreciable reduction in urinary excretion of N-AcAAP but caused significant reductions in free AAP and total AAP. At carbon disulfide concentrations of 20, 40, and 80 ppm (31, 124, and 248 mg/cu m), reductions in free AAP, N-AcAAP, and total AAP were statistically significant. Twelve hours after exposure began, the inhibition had usually reached maximum, after which the levels of AAP and N-AcAAP increased. At the end of the 33-hour observation period, these levels had surpassed preexposure values. After exposure at 10 ppm (31 mg/cu m), free AAP and total AAP excretion levels had returned to

preexposure values by 8 hours after exposure ended. At other concentrations, the times of return to preexposure levels were proportionately longer, being slightly more than 18 hours after the end of exposure at concentrations of 80 ppm (248 mg/cu m).

In the second experiment, amidopyrine administered 18 hours after the 6-hour carbon disulfide exposure was metabolized and excreted in normal time. Carbon disulfide exposures at 20 ppm (62 mg/cu m), 6 hours/day for 5 consecutive days, resulted in greater reduction in AAP excretion than was seen in the single exposure at the same concentration. Mack et al [40] concluded that carbon disulfide, by blocking microsomal mixed-function oxidases, inhibited N-demethylation of amidopyrine, allowing the drug to persist in the body. At the same time, the excretion of the metabolites AAP and N-AcAAP was prolonged. The authors pointed out the possible serious implications of these findings: even very low concentrations of carbon disulfide, 10-20 ppm (31-62 mg/cu m), for 6 hours could retard normal metabolism of such frequently taken drugs as analgesics, hypnotics, antidiabetics, and anticonvulsants. This could be especially important for highly cumulative drugs such as phenylbutazone and hydantoin derivatives. It was suggested that this inhibition of drug metabolism might be a useful indicator of exposure for carbon disulfide-exposed workers. However, because of the short duration of the inhibitory effect, the authors [40] felt that timely urine collection and testing would be difficult.

### Epidemiologic Studies

There are numerous epidemiologic studies of occupational exposure to carbon disulfide in the literature, and these reports constitute the

majority of the biologic evidence associating adverse health effects with exposure to carbon disulfide.

(a) Cardiovascular Effects

Tiller et al [41], in 1968, reported a coronary heart disease mortality study in male viscose rayon workers exposed to carbon disulfide in England and Wales. The authors calculated the percentage of deaths from coronary heart disease in 397 viscose rayon workers who died between 1933 and 1962, for comparison with national rates for the same period. The workers, who were 35-64 years old when they died, had been employed at three viscose rayon factories--223 as rayon process workers (involved in viscose making or spinning and exposed to carbon disulfide, hydrogen sulfide, or both) and 174 as nonprocess workers (not directly exposed to these compounds). The control group consisted of 561 local men of comparable age who died during the same period. In rayon process workers, 42% of all deaths were attributed to coronary heart disease (94 observed versus 42 expected,  $P < 0.001$ ), as were 24% in nonprocess workers (41 observed versus 31 expected,  $P < 0.05$ ) and 17% in the control group (97 observed versus 73 expected,  $P < 0.01$ ). The finding that the proportion of deaths from coronary heart disease in the controls was significantly higher than the national rate (14%), together with the imprecise classification of workers, makes interpretation of the results difficult. Also, monitoring for carbon disulfide was not performed routinely prior to 1945, so that the extent of exposure could not be specified.

Because of these limitations, a second study [41], based on more precise exposure information and work histories, was undertaken at the newest of the three factories. Monitoring for carbon disulfide and



hydrogen sulfide had been performed regularly in this factory after 1945, although frequency and methods of air sampling and analysis were not reported. Carbon disulfide concentrations in the churn rooms of the viscose-making department reportedly exceeded 20 ppm (62 mg/cu m) in 17% of the tests made. Nearly half the air samples from the spinning department had carbon disulfide concentrations above 20 ppm (62 mg/cu m). Workers in the spinning department were exposed to hydrogen sulfide also at concentrations which, although usually low, occasionally exceeded 10 ppm (about 15 mg/cu m). Tiller et al discounted the role of hydrogen sulfide in causing coronary heart disease. They reported that deaths from coronary heart disease in those who had worked exclusively in the viscose-making departments of the two older factories far outnumbered such deaths in the newest, most "modern" factory (13 deaths observed versus 4.6 expected in the older factories and 6 observed versus 5.0 expected in the newest one). Because there was no exposure to hydrogen sulfide in the viscose-making department, the authors attributed the difference in coronary mortality ratios to lower carbon disulfide exposure in the new factory.

Another mortality study in this report [41] was restricted to workers who died after at least 10 years of exposure in the viscose rayon industry, including 1 year or more between 1945 and 1949 at the plant studied. Analysis was limited to the 2,129 employees who were between 45 and 64 years of age at some time during the study period (1950-1964). Records of over 97% of these men were successfully traced and were classified by predominant work experience: viscose making, viscose spinning, or nonprocess; employees of foreman or higher status were divided into spinning and nonprocess staff.

From death certificates, the causes of death were classified as (1) coronary heart disease, (2) other cardiovascular disease, and (3) other causes [41]. Using expected numbers of deaths based on national rates for England and Wales, the authors found that significantly more viscose spinners than expected died of coronary heart disease (28 versus 14.6,  $P < 0.001$ ). Deaths from coronary heart disease were also more numerous than expected in supervisory personnel assigned to the spinning department (9 versus 4.3,  $P < 0.05$ ). Spinning workers with less than 10 years of employment also had a significantly higher than expected incidence of deaths from coronary heart disease (7 versus 2.4,  $P < 0.01$ ). The authors [41] compared the mortality of all men in the spinning department (both operatives and staff) with that of all men working outside the spinning department. The death rates from coronary heart disease were 6.6/1,000 man-years for all men in the spinning room and 2.7/1,000 man-years for all men in other departments. The difference in rates was significant at the 0.01% level of probability. The criteria for classification as "coronary heart disease" were not clearly explained in the study [41]. Because this report was a proportionate mortality study, the relative risks of death from coronary heart disease could have been affected by changes in the percentages of other causes of death, possibly confounding the role of carbon disulfide in causing coronary heart disease. Also, no controls, other than the nationally based "expected" numbers of deaths, were used in the second half of the study. Finally, the sampling and analytical methods used to determine carbon disulfide concentrations were not described.

Hernberg et al [8] studied coronary morbidity and risk factors in male workers exposed to carbon disulfide at a Finnish viscose rayon plant.

Workers aged 25-64 at the time of the physical examination (1967-1968) and those who had died before age 65 were included in the exposed group. All exposed subjects had had at least 5 years of experience between 1942 and 1967 in the departments with the heaviest concentrations of carbon disulfide (primarily the spinning and spinning-bath departments). Of the 410 workers meeting these criteria, 45 had died and 22 others were, for a variety of reasons, not examined. The remaining 343 subjects were examined and matched individually with an equal number of controls who had a minimum of 5 years' continuous work experience at a nearby papermill. The pairings were based on (1) age difference of 3 years or less, (2) proximal birthplaces, and (3) nearly equivalent physical work requirements.

The authors [8] used graphs to report the mean exposure concentrations of hydrogen sulfide plus carbon disulfide from 1945 to 1967. Approximately 3,000 measurements were available, based on 5- to 10-minute air samples taken 1-36 times yearly at 10-40 different sites each time. A titrimetric xanthate method was used to analyze the samples [42]. Roughly, the hydrogen sulfide plus carbon disulfide concentrations were greater than 40 ppm before 1950, 20-40 ppm between 1950 and 1960, and 10-30 ppm in the 1960's. The hydrogen sulfide concentrations were estimated to have been 10% of those of carbon disulfide. An approximate measure of personal exposure was developed for each subject, using an index of "exposure dosage." Despite the limitations of the data, including scanty work histories, possibly nonrepresentative air sampling, and considerable intrafactory mobility, the authors [8] considered that the index gave a reasonably accurate picture of each subject's total exposure to carbon disulfide plus hydrogen sulfide.

In addition to mailing general and coronary history questionnaires to all subjects, the researchers [8] physically examined the subjects. The examination included electrocardiography following measured exercise, chest radiography, and blood pressure measurements. Death certificates were obtained for 43 of the 45 exposed men who died prior to examination and for the 5 who died in the 2 years following the commencement of examinations [8]. No comparable mortality data were obtained for the control group. The authors reported that 52% of the deaths (25 of 48) were from coronary heart disease. The expected percentage, based on official age- and year-adjusted statistics for the male population, was 31.7% (15.2 deaths). This difference was significant at  $P=0.002$  for a two-tailed test. No significant differences were found between the exposed and control groups in their histories of myocardial infarctions. There was a significant difference ( $P<0.05$ ) in the prevalence of angina between the exposed group (17%) and the controls (11%). The clearest difference between exposed and control workers was in blood pressure. The exposed group had a mean systolic blood pressure of 140 mmHg, while that reported for the control group was 136 mmHg ( $P<0.005$ ). For fourth- and fifth-phase diastolic pressures, the differences again were statistically significant ( $P<0.001$ ), the exposed group averaging 4-6 mmHg higher values than controls. While the blood pressure differences were stated to be statistically significant, the clinical significance is questionable because of the known lability of blood pressure. Neither ECG's nor heart-volume data revealed significant differences.

Hernberg et al [8] pointed out the possible "diluting effects" from preemployment screening-out of unhealthy workers, from subsequent employee

transfers and resignations, and from exclusion of patients receiving disability pensions for causes other than carbon disulfide toxicity. The authors emphasized that these factors increased the importance of significant or nearly significant findings as indicators of real effects of carbon disulfide poisoning. Because of good control selection and thoroughness of physical examinations, the authors' conclusions of a possible role of carbon disulfide in coronary heart disease seem valid.

In 1971, the same investigators measured blood lipids, glucose tolerance, and plasma creatinine and cholesterol in the workers previously studied [8]. From all 343 viscose rayon workers and their matched controls, the authors [43] drew fasting venous blood samples. Glucose was given orally to the subjects as a 10% solution in amounts proportional to their body weights, and postload blood samples were drawn 1 and 2 hours later. No significant differences were found between the total exposed and control groups or any of the three paired subgroups for plasma cholesterol, serum triglycerides, serum free fatty acids, total serum lipids, or plasma glucose values. The mean fasting glucose value of subjects with more than 15 years' exposure and the 1-hour value of the entire group were very nearly significantly elevated above control values ( $0.05 < P < 0.10$ ). The only parameter that showed a significant effect from carbon disulfide dose or duration of exposure was the fasting glucose concentration, which was correlated with both the exposure duration and the exposure index ( $P < 0.05$  in both cases). The mean plasma creatinine for 68 exposed men was 1.00 mg% versus 0.94 mg% for the controls ( $P = 0.02$ ). This indicated a tendency toward increases in fasting blood glucose and creatinine concentrations after prolonged carbon disulfide exposure.

In a 5.5-year followup study published in 1973, Hernberg et al [44] reported excess coronary heart disease in the cohort used in their earlier study [8]. Mortality in the exposed group of 343 workers was compared with that of their corresponding controls in the 5.5 intervening years [44]. However, evaluation of the effectiveness of the matching scheme revealed that the responses of paired exposed and control workers were not correlated; hence, the exposed and control data were treated as two independent series. Of 23 deaths in the exposed cohort, 16 were from coronary heart disease, as were 3 of 9 deaths in controls during the 5.5-year followup. The relative risk of exposed workers versus controls was 5.6 for coronary heart disease mortality and 2.7 for overall mortality. Calculations from the authors' data, however, yielded values of 5.2 and 2.6. The relative risk is defined as the ratio of the rate of disease in those exposed to the rate in those not exposed. Both ratios differed significantly from 1.0. The mortality rate from coronary heart disease in the exposed group was 4.7% and the overall mortality rate was 6.7%, while the corresponding rates in the control group were 0.9% and 2.6% for the 5.5-year followup period. Therefore, the attributable risk for coronary heart disease mortality among viscose rayon workers exposed to carbon disulfide was 3.8% and that for overall mortality was 4.1%, with probabilities of 0.002 and 0.011 that the attributable risks differed from zero. The attributable risk is defined as the difference in disease rates between those exposed and those not exposed.

Based on the total Finnish male population, the relative risk of coronary heart disease was greater than 1.0 for 45- through 69-year-old exposed workers in each of the 5-year age groupings [44]. The relative

risk for all ages was 2.05 ( $P < 0.01$ ). The age-adjusted total relative risk was 2.10. The relative risk of the nonexposed cohort was 0.37, indicating a smaller than expected number of deaths from coronary heart disease in the control group as compared with the general male population ( $P < 0.05$ ). This result implies an inherent selection of healthy individuals in the industrial environment.

Using discriminant function analysis (a multivariate technique used to differentiate populations), the Hernberg group [44] attempted to assess the quantitative effect of carbon disulfide exposure in relation to other variables of potential risk. In addition to carbon disulfide exposure, calculated for each subject as an individual exposure index [8], they considered the four risk factors of age, history of cigarette-smoking, diastolic blood pressure, and serum cholesterol level [44]. The discriminant function classified the exposed workers as to those who would die of coronary heart disease and those who would die of other causes. The function proved accurate for 88.7% of the workers who died. The mean values of each of the five risk factors for those who died of coronary heart disease were significantly different ( $P < 0.05$ ) from those for workers who died of other causes. Nevertheless, the importance of carbon disulfide exposure as a risk factor in coronary heart disease mortality was not obvious from this analysis. The authors therefore graphed the risk ratio functions at various exposure levels by age. The absolute risk of death from heart disease increased with age, but, with adjustment for age, carbon disulfide exposure became the more important factor. Hernberg et al [44] stated that, although not precise, the exposure data they had reported earlier [8,43] had become the basis for the lowering of the Finnish

threshold limit value (TLV) from 20 to 10 ppm (62 to 31 mg/cu m) in 1972. Such data cannot prove causation of coronary heart disease by carbon disulfide exposure; however, these data suggest a strong association between exposure to that chemical and coronary heart disease.

An additional 5-year followup to the Hernberg et al [8] study on workers exposed to carbon disulfide was reported in 1975 by Tolonen et al [45]. Between 1967 and 1972, 11 of the 343 exposed workers and 4 of the 343 nonexposed workers survived a first myocardial infarction. During the same period, there were 14 fatal attacks in the exposed group and 3 in the control group, giving a total of 25 myocardial infarctions in exposed workers and 7 in nonexposed workers. The incidence rate was 7.5 infarctions/100 workers in the exposed group and 2.1 in the control group (significant difference at  $P=0.0012$ ), giving a relative risk of 3.6, although the authors reported this ratio as 3.7. As in the original study [8], the ECG tracings revealed abnormalities in the exposed group, but these were not significantly different from those seen in tracings from the controls [45]. For workers exposed to carbon disulfide, the relative risk was greater than 1.0 for "typical angina," as well as for probable angina and possible angina. Although probable angina showed the highest relative risk and associated significance in the original study [8], Tolonen et al [45] found in the followup that typical angina showed the highest relative risk (2.8), with a prevalence rate of 12.0% versus one of 4.9% in the control group ( $P<0.001$ ). Symptoms of angina were nearly twice as frequent in workers exposed to carbon disulfide as in the control group (24.6% versus 13.0%, significantly different at the 0.0001 level). Summarizing coronary heart disease manifestations, Tolonen and coworkers [45] reported



that the more severe the outcome, the more evident the relationship to carbon disulfide exposure. The relative risks were 4.8 for fatal infarctions, 3.6 for all infarctions, 2.8 for nonfatal infarctions, 2.2 for angina, and 1.4 for "coronary ECG's." In terms of attributable risk, angina symptoms prevailed with 11.6% because of the greater frequency of angina than of more severe symptoms.

Tolonen et al [45] also reported that, as in the original study [8], blood pressures were significantly higher in the exposed group. They found a maximum difference of 17% between cumulative frequency-distribution curves for systolic blood pressure of exposed workers versus controls ( $P < 0.001$ ) [45]. The difference was 14% for diastolic pressure ( $P < 0.01$ ). Compared to controls, exposed workers showed a slight, nonsignificant increase in relative heart volume.

To study risk factors in infarction cases, Tolonen et al [45] matched a group of 32 exposed and unexposed men who had had myocardial infarctions during the followup with 32 exposed and unexposed men free from the condition. The exposed men had significantly higher systolic and diastolic blood pressures and a history of more cigarette smoking than the controls ( $P < 0.001$ ,  $P < 0.02$ , and  $P < 0.01$ , respectively). The authors [45] did not find a significant relationship between exposure duration and incidence of coronary infarction.

Tolonen et al [45] concluded that the highest relative risks were evident in the most severe myocardial attacks and the lowest relative risks (although larger than 1.0) in the mildest forms of coronary heart disease (eg, anamnestic angina, "coronary ECG's"). Accordingly, they suggested that carbon disulfide may cause a worsening prognosis of coronary heart

disease at a given severity, while perhaps also slightly increasing the incidence and prevalence of symptoms related to coronary heart disease. Tolonen et al [45] related their clinical data to workroom concentrations and to the TLV for carbon disulfide. The concentrations of hydrogen sulfide plus carbon disulfide during the 10 years prior to this study had generally been near or below 20 ppm. Sampling, which was done only monthly and only under "normal" conditions, may have failed to detect peak exposure and no-exposure times. More important, by 1968, 38% of the workers were no longer exposed to carbon disulfide, and about 40% were not exposed in the followup period (1968-1972). This, the authors concluded, indicated irreversible, deleterious effects of carbon disulfide on the development of coronary heart disease, and they suggested that the recently observed levels of 10-30 ppm were too high to safeguard employee health. However, the Tolonen group [45] did not discuss the possible role of hydrogen sulfide.

In 1976, Hernberg et al [46] reported on an 8-year followup to their studies on coronary heart disease mortality in viscose rayon workers. Of the 343 original members of the exposed cohort assembled in 1967, 165 men (48%) were still employed in the plant in 1975, but only 64 (19%) were still exposed to carbon disulfide. All workers were accounted for in the followup study. Marked decreases in the concentrations of carbon disulfide plus hydrogen sulfide were noted during the final 3 years of the followup: concentrations were around 5 ppm in the rayon staple-fiber factory and less than 5 ppm in the rayon-filament factory. The 8-year incidence rate of coronary mortality was 5.8% in exposed workers and 2.6% in controls. Total mortality rates for the two groups during the same period were 10.2% and

6.7%. The difference in coronary mortality was 3.2%, and the difference in total mortality was 3.5%, indicating that coronary heart disease was almost totally responsible for the excess mortality. However, during the last 3 years of the followup, six deaths attributed to coronary heart disease occurred in each cohort. Because only 19% of the original exposed cohort remained exposed to carbon disulfide in 1975 (versus 53% in 1972), and because environmental concentrations had greatly decreased by 1975, the authors [46] stated that the decrease in coronary mortality coincided with the changed conditions. Hernberg et al [46] concluded that this apparent reversibility suggested that the previously reported excess coronary mortality may have been caused by direct toxic effects of carbon disulfide on the myocardium rather than to an acceleration of the atherosclerotic process. The authors, however, based this "reversibility" theory on only 3 years of followup and six coronary heart disease deaths in each cohort during this period. Further, this finding in no way contradicts earlier findings of excess coronary mortality [8,44,45], because improved work practices, administrative controls, and extensive use of personal protective equipment had markedly improved working conditions. It should be noted, however, that increased mortality in the control group during the last 3 years of this 8-year study contributed much more to the eventual equality of mortality rates of the exposed subjects and the controls than did decreased mortality in the exposed group during the same period.

Nurminen [47], in 1976, analyzed the 8-year survival experience of the cohort of 343 viscose rayon workers developed by Hernberg et al [8] in 1967. The author developed a life table to study life-expectancy differences and calculated age-specific death rates from coronary heart

disease. The 8-year cumulative incidence rates of coronary heart disease found by Nurminen [47] for the exposed and control groups were 5.8% and 2.6%, and the relative risk was 2.22. A two-tailed confidence interval revealed that the relative risk was significantly larger than 1.0. The attributable risk percent was 55%. Overall mortality data also showed increased risk in exposed workers. The estimated 8-year survival rate from coronary heart disease was 94.1% for the exposed workers and 97.3% for the controls, based on the changing structure of the cohorts. In all age groups (25-44, 45-49, 50-54, 55-59, 60-64, and 65-72), the unexposed workers had a longer life expectancy, with a range of 0.9-2.1 additional years. At older ages, the absolute differences in life expectancy decreased but the percentage differences increased. The author also found that the incidence rate increased markedly until age 65. Death rates from coronary heart disease in exposed workers were higher than those for the controls after age 50 (except age 65-72). Although coronary mortality during the entire 8-year followup period was higher in the exposed group, coronary mortality was lower in the exposed group during the 8th year of the followup. Nurminen [47] concluded that, because environmental and administrative modifications were instituted in the factory during the final 3 years of the followup, the risk of coronary death in formerly exposed viscose rayon workers could have been lowered by decreased carbon disulfide concentrations.

Cirila et al [48] studied 325 rayon factory workers in Italy to determine the relationship between risk of coronary heart disease and occupational exposure to carbon disulfide. Only workers with at least 5 years of exposure to carbon disulfide and without any history of coronary

disease prior to employment were included. The workers were divided into three groups: 125 subjects exposed to carbon disulfide at average concentrations equal to or higher than 120 mg/cu m (39 ppm); 28 subjects exposed at concentrations averaging 60 mg/cu m (19 ppm); and 172 unexposed workers. All subjects were men of similar backgrounds with equivalent job-related workloads. The moderately exposed group (only 28 individuals) had a larger percentage of smokers (93%) than either the heavily exposed group (71%) or the control group (73%); however, the percentages of heavy smokers (more than 10 cigarettes/day) were similar in the three groups (29% for heavily exposed workers, 32% for moderately exposed workers, and 34% for controls). ECG's at rest and after a Master's two-step test with the 12 conventional leads were made after the subjects had fasted for at least 2 hours. Two cardiologists interpreted the ECG's, using supplemented patient medical history data, in a double-blind design. In cases of discordant diagnoses, the two cardiologists discussed the findings to reach agreement.

The authors [48] found ECG patterns indicating coronary artery disease in 5.6% of the subjects exposed to carbon disulfide at average concentrations above 120 mg/ cu m (39 ppm), in 3.6% of those exposed at 60 mg/cu m (19 ppm), and in 1.2% of the controls. There was a trend toward increased arterial hypertension with increased carbon disulfide exposure. A "trend" chi-square test showed significance ( $P < 0.05$ ) in the increase in coronary heart disease as a function of carbon disulfide exposure. Also, a 2 x 2 chi-square analysis of coronary heart disease in the high-exposure group versus that in the control group showed a significant difference ( $P < 0.05$ ). The relative risk of coronary heart disease for highly exposed workers compared with that of controls, calculated from the authors' data,

is 4.8. Although causation was not proven at concentrations around 60 mg/cu m (19 ppm) (the present Italian MAC), the authors considered the MAC for carbon disulfide to be too high to protect against the development of chronic pathogenic coronary heart disease development.

Gavrilescu and Lillis [33] examined 138 artificial-silk workers whose exposure to carbon disulfide, averaging more than 10 years, had been at concentrations averaging between 20 and 42 mg/cu m (6-13 ppm) during the past 8 years, with peaks of 120-180 mg/cu m (39-58 ppm); earlier levels, believed to have been higher, were not documented. Atherosclerotic changes, as indicated by clinical, electrocardiographic, oscillometric, and optic fundi examination and by estimations of cholesterolemia, triglyceridemia, and lipidemia, were found in 30.4% of the subjects and arterial hypertension in 23.2%; 14.5% of the workers showed both conditions. No control group was studied, but the authors stated that these percentages were significantly higher than in comparable groups of the population not exposed to carbon disulfide. Oscillometry disclosed that many of the atherosclerotic changes occurred in the main arteries of the legs; others were in the cerebrum; and a few workers showed ECG signs of coronary atherosclerosis. Hypertension was more frequent in workers with more years of exposure; 50% of the workers exposed to carbon disulfide for more than 20 years had arterial hypertension. The authors warned against exposing employees with a familial history of arterial hypertension, diabetes mellitus, obesity, or atherosclerosis to carbon disulfide. This report did not contain specific methodologies of air sampling and analysis or diagnosis of the conditions found, nor did it give adequate data to substantiate its results.

Locati et al [49] studied 116 cases of chronic carbon disulfide poisoning diagnosed in exposed viscose rayon workers who were admitted to the Clinica del Lavoro of Milan between 1947 and 1969 for possible occupational disability compensation. All subjects had been exposed to carbon disulfide at concentrations averaging above 20 ppm (62 mg/cu m) for more than 5 years, although exact concentrations were not known. The mean age of the workers was 50.4 years at the time of examination. The prevalence of coronary heart disease was studied by examining the clinical and ECG data on each subject and by using Goldberger's [50] criteria for diagnosis. The affected viscose rayon workers were compared with 120 men (mean age 51.2 years) admitted to the clinic for suspected silicosis but found to be unaffected by lung disease [49]. Although more viscose rayon workers were diagnosed as affected by coronary heart disease, the difference was not statistically significant. However, using 111 workers referred to the clinic for suspected carbon disulfide poisoning but found to be unaffected as controls, Locati et al [49] noted that 16.5% of the poisoned workers versus 2.7% of the controls had coronary heart disease. A chi-square test with their data shows a difference significant at the 0.001 level. Because of lack of specificity in selection of controls, these results can be regarded only as weak evidence for a role of carbon disulfide in the development of coronary heart disease. Because coronary heart disease in viscose workers was not compensable in Italy and only those workers with compensable disabilities were sent to the Clinica del Lavoro, it is possible that workers with coronary heart disease were admitted to other hospitals. This suggests that there may have been even larger differences between poisoned workers and controls in the incidence

of coronary heart disease. The lack of details concerning individual exposure levels did not permit reliable correlation of dose and effect.

Lieben et al [51], in 1974, studied the cardiovascular effects of carbon disulfide exposure in 1,498 male viscose rayon workers in the United States. All workers were 45 years of age or older and had 10 or more years of service at any of three plants. Although participation was voluntary, 87%, 97%, and 100% of the workers at the three plants participated in the study. The controls were 481 acetate plant workers. The survey consisted of obtaining an ECG (read "blind" by a certified cardiologist), blood pressure, total cholesterol, and height, weight, age, and occupational exposure history for each employee. The employees were divided into four study groups on the basis of exposure history: group 1 had no carbon disulfide exposure (acetate workers); group 2 had "possible" exposure (occasional but not constant exposure); group 3 had "intermediate" exposure (viscose department workers for more than 10 years and workers with less than 10 combined years in spinning and staple departments, but with a total of 10 or more years in the viscose plants); group 4 had "heavy" exposure (more than 10 years of experience in staple or spinning departments). Specific environmental concentrations of carbon disulfide were not reported.

The authors [51] reported that the only statistically significant finding was that hypertension and borderline hypertension were more frequent in the exposed-worker groups than in the controls. Hypertension was defined as systolic pressure of 160 mmHg or more or diastolic pressure of 95 mmHg or more. The percentages of hypertensives were 18, 28, 27, and 30% for the control, "possible," "intermediate," and "heavy" exposure



groups, respectively. Borderline hypertension (defined as systolic pressure of 140-160 mmHg or diastolic pressure of 90-95 mmHg) or hypertension was found in 38, 60, 58, and 60% of the four exposure groups. A test for trends from lowest to highest exposure group revealed a significant trend in hypertension, with or without borderline cases. Although other trends were significant, the authors felt that it was "reasonable not to pay any particular attention" to them. They concluded that the hypertension problem was the only significant finding of the study, even though the differences between exposed and control workers in mean blood pressures were slight (140/87 mmHg versus 135/83 mmHg).

The authors [51] mentioned three shortcomings in their paper: the problems inherent in using a survivor group; the inexact evaluation of employee exposures to carbon disulfide; and the limitations of a retrospective study. They doubted the importance of employee turnover during the period of observation because of the advantages earned by seniority (eg, better wages, more security). However, they reported that 96 workers aged 45-64, with more than 10 years' experience, left one of the plants in 1972. Of these, 12 retired for health reasons and 11 died. If some of these 23 disabilities and deaths were cardiovascular in nature, the data and conclusions of this study could definitely have been altered. Another problem in this study was the use of acetate workers as the control group. Because hypotension has been associated with exposure to acetone [52,53], a different control group would have been more appropriate.

Kramarenko et al [54] examined 94 young women, aged 17-19 years, who underwent vocational training for 9 months at two viscose rayon mills. The two plants reportedly used basically the same technology and had similar

working conditions. The 44 women working at Mill A were reported to be exposed to carbon disulfide at average concentrations of about 20-30 mg/cu m (6-10 ppm). The 50 women at Mill B were reported to be exposed to carbon disulfide at average concentrations of 3-10 mg/cu m (1-3 ppm). Hydrogen sulfide concentrations did not exceed 10 mg/cu m (3 ppm) at either mill. The workers were given medical examinations at the beginning of training and after 5, 7, and 9 months. Cardiovascular, muscular, and nervous system functions were examined. All systems showed functional modifications during the 9 months of training. The mean systolic blood pressure of Mill A trainees, after an initial rise from 103.0 to 110.1 mmHg at 5 months, decreased to 93.6 mmHg after 9 months ( $P < 0.001$ ). Mill B trainees did not show a significant decrease in systolic blood pressure, but both groups showed significantly decreased diastolic pressures (Mill A: 64.1 to 47.6 mmHg,  $P < 0.001$ ; Mill B: 62.1 to 53.0 mmHg,  $P < 0.05$ ). Pulse rates were also significantly decreased for both groups ( $P < 0.001$ ) after 9 months of training (Mill A: 83.0 to 71.6 beats/minute; Mill B: 78.9 to 74.7 beats/minute). A shorter latency period for simple and complex reactions of the nervous system was found in both groups. The findings of hypotension and nervous system excitability [54] indicate the opposite of other reports which showed hypertension and slower neuromuscular reaction times [55-57]. The fact that the subjects were very young and female may have had some bearing on the results, although this remains uncertain.

(b) Effects on the Reproductive System

Lancranjan et al [58], in 1969, studied testicular changes in young workers in an artificial-fiber factory who had been exposed to carbon disulfide at average concentrations of 40-80 mg/cu m (13-26 ppm), with

peaks up to 780 mg/cu m (250 ppm). On the basis of clinical, biochemical, vascular, and electromyographic (EMG) examinations, the 33 workers had been diagnosed as chronically poisoned by carbon disulfide. The workers had a mean age of 22 years and a mean length of exposure of 21 months. The 31 controls had a mean age of 25.9 years. The patients were given endocrinologic examinations, using urine and semen analyses. Disturbances of "sexual dynamics" were observed in 78% of the patients, decreased libido (66%) and erection difficulty (51%) being the most common problems. Semen analysis revealed that the poisoned workers had significantly higher frequencies than the 31 controls of asthenospermia (18 versus 3 cases,  $P < 0.001$ ), hypospermia (11 versus 3 cases,  $P < 0.025$ ), and teratospermia (25 versus 4 cases,  $P < 0.001$ ). The excretion of total neutral 17-ketosteroids was lower in the exposed workers than in the controls. However, this decrease was not correlated with duration of exposure. The authors [58] suggested that carbon disulfide may act both on the hypothalamus and directly on the gonads to produce the observed effects. It should be noted that the authors first stated that there were 33 exposed subjects with a mean age of 22 years, but later mentioned 32 subjects with a mean age of 25 years.

In 1972, Lancranjan [59] examined 133 chronically poisoned male viscose rayon workers and 50 male controls, both with an average age of 30 years, for spermatid disorders. Using methods described in a previous study [58], Lancranjan [59] again found significantly increased frequencies of hypospermia, teratospermia, and asthenospermia in poisoned workers over controls.

Vasilyeva [60] studied female viscose rayon workers in three different departments for possible effects of carbon disulfide on ovarian function and menstruation. The study included 500 workers in the spinning shop, where carbon disulfide concentrations sometimes exceeded 20 mg/cu m (6 ppm) and hydrogen sulfide concentrations reportedly never exceeded 10 mg/cu m (7 ppm); 209 workers in the trimming department, where the concentration of neither carbon disulfide nor hydrogen sulfide exceeded 10 mg/cu m (3 ppm); and 429 workers in the rewinding-sorting department (controls), not exposed to either substance. Durations of menstrual flow of more than 5 days occurred in 17.8% of the spinners, 10.5% of the trimmers, and 5.1% of the controls ( $P < 0.001$ ). Workers in the spinning shop experienced irregular menstruation significantly more frequently than the controls (7.6% and 1.6%, respectively;  $P < 0.001$ ). The frequency of irregular menses increased with longer occupational exposure. Heavy menstrual flow occurred in 12.5% of the spinners, 11% of the trimmers, and 2.3% of the controls ( $P < 0.001$ ); painful menstruation was also significantly more common in exposed workers (36% and 38%) than controls (17%). These disorders increased in frequency with increased job longevity. Finally, 48 women from the spinning shop, 29 from trimming, and 35 from the rewinding-sorting shop were examined for cellular disturbances in vaginal smears at various times during the menstrual cycle. Twelve of 48 spinners and 11 of 29 trimmers, but only 3 of 35 rewinding-sorting workers, had cellular changes. A biochemical study of the sex hormones in the urine confirmed the vaginal-smear findings, and the authors concluded that women working in the spinning and trimming departments had disturbances in ovarian hormone production. This report suggests that higher levels of carbon disulfide

and longer exposure durations may lead to more pronounced ovarian disturbances. A possible dose-response relationship for carbon disulfide is suggested from these data, since hydrogen sulfide concentrations were approximately equal for both exposed groups but effects were more frequent in the groups exposed to the higher level of carbon disulfide.

Petrov [61] analyzed pregnancy data for 380 women employed in the viscose industry to determine the effects of carbon disulfide on pregnancy. The exposed group included 189 women who, before and during pregnancy, were exposed to carbon disulfide at concentrations reported to be 2.7 times the Soviet permissible limit of 10 mg/cu m (3 ppm). These women worked in the viscose-spinning shops. The group of 191 controls had not been exposed to carbon disulfide but had worked under similar conditions in the same factory. The women in the control group were somewhat younger than the exposed women; 63% of the exposed women were between 20 and 30 years old, versus 84.8% of the controls. Exposed women had worked slightly longer than controls; 91.0% of exposed women had worked longer than 3 years versus 84.8% of the controls. Of the exposed women, 62.8% were primiparous (ie, had had one pregnancy), versus 65% for controls. Several pregnancy complications were recorded, and comparisons were made between exposed and control women. The rate of threatened pregnancy terminations in the exposed group was 25.9/100 pregnant women versus 13.1/100 pregnant women in the controls ( $P < 0.05$ ). The difference was still significant after adjustment for the differences in age and job longevity. Threatened pregnancy terminations occurred more frequently in the exposed women than in the controls, 12.5% versus 9.4% in the 20- to 24-year-old age group and 35.4% versus 13.6% in the 25- to 29-year-old age group. Spontaneous

abortions occurred in 14.3% of the exposed women and 6.8% of the controls ( $P < 0.05$ ). Differences were still significant after age and job-longevity adjustment. A correlation coefficient did not indicate any dependence of the rates of spontaneous abortion on length of exposure. The correlation coefficient for threatened miscarriage and length of exposure was also not significant. Significantly more exposed women gave birth prematurely than did controls (8.6% versus 2.8%). Petrov [61] concluded that elevated carbon disulfide concentrations decreased the probability of bringing a pregnancy to term. The findings of this report may be important indicators of carbon disulfide toxicity, especially in consideration of the low concentrations of carbon disulfide reported (approximately 27 mg/cu m or 9 ppm) if these concentrations are representative of actual workplace exposures.

Bezvershenko [62], in 1965, reported on the influence of occupational exposure to carbon disulfide on menstrual and reproductive function in 206 female viscose production workers. The viscose production workers were predominantly 25-38 years old, and over 50% had more than 10 years of service in the industry. Workplace concentrations of carbon disulfide and hydrogen sulfide were not given. No data on the occupations, age, or length of employment of the 60 control subjects were given. Examinations revealed that 22.3% of the women in the experimental group and 8.3% of the controls had various ovarian and menstrual cycle disorders arising during employment which were not attributable to other conditions or diseases. He found dysmenorrhea in 7.3% of the exposed women and 1.6% of the controls, irregular menstruation in 6.8% of exposed women and 3.3% of controls, and delayed menstruation in 9.2% of exposed workers and 3.3% of controls.

Oligomenorrhea and other menstrual difficulties occurred more frequently in exposed women than in controls. Spontaneous abortions were reported to have occurred in 8.7% of the exposed women and 3.3% of controls. Long-term inability to conceive was reported in 13.6% of the exposed women and in 5% of the controls. Bezvershenko [62] reported that these disorders occurred predominantly in workers in the spinning shops and specifically in those workers with many years of service. Urine-excreted estrogens were measured in 19 hospitalized subjects. Seventeen showed elevated quantities of estrogens, with a range of 377.5-940.7  $\mu\text{g}$ ; the normal range was considered to be 50-300  $\mu\text{g}$ . Vaginal smears also indicated disorders of ovarian function. Because there are inadequate data on experimental procedures, exposure concentrations, and the constitution of the control group and no statistical analyses of the data, this report can be considered to be only a qualitative description of menstrual and reproductive dysfunction in female viscose production workers.

(c) Neurologic Effects

Hanninen [63] studied workers from a viscose rayon factory for possible psychological and behavioral disorders. Three 50-man groups were designated: carbon disulfide-intoxicated workers, workers exposed to carbon disulfide for at least 5 years but without clinical symptoms, and unexposed workers. The average monthly carbon disulfide concentrations recorded in the contaminated areas of the factory were 30-90 mg/cu m (10-29 ppm) in the 1960's, with higher levels earlier. Of the 50 carbon disulfide-intoxicated workers (determined on the basis of clinical, neurologic, otoneurologic, and neuroophthalmic examinations), 33 were still working in the factory. All men in the exposed but nonintoxicated group

considered themselves healthy and reported only mild and transient symptoms, eg, headache, insomnia, pain in the limbs. Four men in the control group had had temporary symptoms of carbon disulfide poisoning in the past during "casual work in contaminated areas." Significant differences between the intoxicated and control groups were observed in performance on tests involving speed, vigilance, manual dexterity, and intelligence. On psychomotor and visual performance tests, test scores of nonintoxicated workers were closer to those of the intoxicated group than to those of the controls. The author [63] considered this important because it indicated that carbon disulfide exposure had affected workers who showed no clinical symptoms. Some of the typical signs and symptoms of the exposed workers were poor visual performance, impaired dexterity, and disturbances in manual coordination and psychomotor behavior.

Tuttle et al [64], under contract to NIOSH, examined a group of US viscose rayon workers for possible behavioral and neurologic disorders. Motivated largely by the work of Hanninen [63], Tuttle et al [64] selected a test battery to screen viscose rayon workers. Psychologists and project staff with experience in behavioral toxicology recommended the tests to be administered. Among the variables measured were fatigability, memory, perception, attention, concentration, visual and neurologic performance, manual dexterity, reaction time, and color vision. Participants were recruited from a viscose rayon plant by local union representatives with guidance from the researchers. Workers currently in jobs with daily exposure to carbon disulfide were selected first, followed by those who had recently left daily-exposure jobs. Because management cooperation was not obtained in this study, the researchers were not able to monitor for carbon



disulfide in the workplace. However, a 1973 NIOSH Health Hazard Evaluation and Determination Report [9] conducted at the same plant found TWA concentrations ranging from 4.3 to 129 ppm (13.3 to 400 mg/cu m), with peaks of more 2,000 ppm (6,200 mg/cu m). This report is discussed in detail in Chapter IV.

The control group in the study by Tuttle et al [64] consisted of rayon factory workers who had not been exposed to carbon disulfide. However, because the desired sample sizes (100 exposed and 50 control workers) could not be obtained exclusively from rayon-plant union personnel, subjects from a local carpenters' union (2 exposed and 14 unexposed) and from the viscose rayon plant management (23 exposed and 6 unexposed) were added to the 89 exposed and 5 unexposed members of the viscose rayon union. A total of 114 exposed workers and 25 unexposed workers (in addition to 2 workers who had uncertain exposure histories) were selected for study. The exposed subjects had a mean age of approximately 40 years. The mean years of exposure for the exposed group was 8.4; the standard deviation, however, was greater than the mean. Work histories, iodine-azide urinalyses, neurologic, and behavioral test data were collected for each subject. Polyneuropathy was indicated in 12 subjects, using a Total Neurologic Score based on physical examination and electrodiagnostic testing. To determine the relationship of carbon disulfide exposure to medical and neurologic findings, correlation analyses were performed. Product-moment coefficients were calculated from exposure indices and neurologic variables, as were partial coefficients (holding the effect of age constant). Statistically significant positive correlations were found for the electrodiagnostic scores and total neurologic scores,

and significant negative coefficients were calculated for nerve conduction velocities. However, the partial coefficients showed smaller correlation values, only one being significant. A significant positive correlation was found between postshift iodine-azide exposure coefficients and neurologic rating scores. Neither signs, symptoms, nor electrodiagnostic scores significantly correlated with preshift or postshift iodine-azide values.

Tuttle et al [64] found significance in nearly all the correlation coefficients of exposure versus behavioral test scores. The partial coefficients (again holding the effects of age constant) demonstrated that exposure indices correlated positively with the degree of behavioral impairment, as measured by the psychological tests. There were also several statistically significant relationships between behavioral test scores and neurologic variables. When age was held constant, the partial correlations were smaller, and fewer were significant.

The authors [64] attempted to replicate the findings reported by Hanninen [63] in 1971. Tuttle et al [64] analyzed differences in several variables in four groups classified on the basis of neurologic examinations. Group 1 consisted of those with evidence of abnormalities, group 2 had possible abnormalities, group 3 had been exposed but had no signs of abnormality, and group 4 was composed of workers who were unexposed and without abnormalities. Simple comparison of descriptive variables revealed that group 1 workers were approximately 20 years older than those in the other groups. Blood pressure and years of exposure were also higher in group 1 workers. Analysis of variance was performed on test scores for the four groups. Many differences were found among the four groups, and several significant differences were found when group 1 scores

were compared with those of groups 3 and 4 (the "control" group). No significant differences were found between group 2 and group 3 and group 4. Analyses of covariance, using age as the covariate, revealed that although age was a factor in creating group differences, most tests still showed significant differences among the four groups.

Tuttle et al [64] concluded that exposure to carbon disulfide was significantly related to both indices of neurologic health and to behavioral test scores. There were also significant relationships between behavioral test scores and indices of neurologic health. These results indicate that the use of behavioral tests to detect preclinical signs of carbon disulfide poisoning may be of value.

Seppalainen et al [55], in 1972, reported on the examination of 36 male viscose rayon workers, diagnosed as chronically poisoned by carbon disulfide, for indications of clinical, neurophysiologic, and psychologic abnormalities. The workers were diagnosed as poisoned on the basis of examinations by a clinician, a neurologist, and a psychologist. The mean age of the group at the onset of intoxication was 36 years; the mean age at the time of this study was 42 years (patients over 60 were not included in the study). The control group consisted of 188 papermill workers not exposed to carbon disulfide. The concentrations at which the viscose rayon workers were exposed were 10-30 ppm (31-93 mg/cu m) in the 1960's, 20-40 ppm (62-124 mg/cu m) in the 1950's, and higher than 40 ppm (124 mg/cu m) prior to 1950. The most significant differences between the poisoned and control groups were the prevalence of general fatigue, insomnia, paresthesia, and headaches in the exposed workers ( $P < 0.001$  for all four symptoms). Psychologic testing revealed mild intellectual impairment,

reduction of sensorimotor speed, and impaired psychomotor ability. The psychologic disturbances were said to correlate well with duration of exposure, ie, patients with shorter carbon disulfide histories generally had milder disturbances.

Seppalainen et al [55] found sensory, motor, or sensorimotor effects in 26 of the patients. One-third of the patients had cranial nerve lesions (eight with acoustic disorders, six with trigeminal sensory neuropathy, five with facial weakness, and one with a bilateral olfactory lesion). Extrapyrarnidal disorders occurred in 14 patients. Muscular weakness was found in 6 patients, muscular "wasting" in 12, and myastheniform symptoms in 6. The 36 patients were divided into 3 categories according to the length of time elapsed since diagnosis of poisoning. Workers in Group A had been diagnosed 0.5-2 years previously; those in Group B, 3-10 years previously; and those in Group C, more than 10 years previously. Of the nine chronically poisoned men in Group A, two had normal conduction velocities (CV's), and two others had normal electromyograms (EMG's). In Group B, 11 of 17 patients had normal CV's, and 4 had normal EMG's. Among the 10 patients who had stopped working with carbon disulfide more than 10 years previously, 4 had normal CV's, and only 1 had a normal EMG. The limits of normality were drawn from test results of 120 "normal" adults (20-60 years old). The most frequent EMG abnormality was a decreased number of motor units in maximal contraction. This was generally associated with neurogenic muscular weakness. The authors [55] concluded that these tests demonstrated that carbon disulfide poisoning caused disturbances in both the peripheral and central nervous systems. They suggested that diminished CV's indicated polyneuropathy, EMG abnormalities

generally indicated damage at the spinal cord level, and myasthenic fatigability indicated a disturbance at the myoneural junction. However, it was emphasized that only 36 men were included in the study. Moreover, significance tests were applied only to the clinical observations; therefore, the other results must be regarded as descriptive. Finally, these results must be considered to be exaggerated evidence of the effects of occupational exposure to carbon disulfide because the subjects were included in this study on the basis of their diagnosis as chronically poisoned.

Seppalainen and Tolonen [56], in 1974, compared 118 male viscose rayon workers, drawn from the same group studied by Hernberg et al [8], who had been exposed for a mean of 15 years with 100 papermill workers (controls) for possible neurophysiologic differences. No subjects, either exposed workers or controls, were eliminated from the study on the basis of present or past histories of neuropathy, as carbon disulfide-induced neuropathy was indiscernible from that from other causes. The greatest difference between exposed and control workers was found in the conduction velocities of the slower motor fibers in the ulnar nerve (39.8 versus 44.1 m/second,  $P < 0.0005$ ) and the deep peroneal nerve (35.5 versus 38.2 m/second,  $P < 0.0005$ ). Significant differences from normal were also found in the maximum motor conduction velocities (MCV) of the posterior tibial nerve (40.5 versus 42.4 m/second,  $P < 0.005$ ) and deep peroneal nerve (45.9 versus 47.3 m/second,  $P < 0.0025$ ). A conduction velocity was determined for each nerve tested such that 5% of the controls showed a CV below this value; each subject was then assigned a total CV score by counting one point for each nerve whose CV was below the limit for that nerve. The distribution

of scores showed significantly slower conduction velocities in exposed workers. The authors regarded lower CV scores as an indication of increased polyneuropathy. The exposed group also had a larger number of abnormal EEG's (21 of 54) than did the controls (6 of 50); this difference was significant at the 1% level. Because the cessation of exposure to carbon disulfide did not change the decreased CV's, the authors [56] concluded that the observed subclinical polyneuropathy may have been irreversible. These findings, while statistically significant, do not clearly indicate that carbon disulfide is the causative factor. The role of hydrogen sulfide, which was measured in combination with carbon disulfide, has not been clearly differentiated.

Vasilescu [57] measured peripheral nerve conduction velocity (CV) and muscular contraction potential (measured by EMG) in workers suspected of having polyneuritis from carbon disulfide exposure. Sixty synthetic-fiber factory workers who had been exposed to carbon disulfide at concentrations of approximately 15 mg/cu m (5 ppm), with peaks occasionally as high as 700 mg/cu m (225 ppm), were examined. Conduction velocities were measured in the median, cubital, and peroneal nerves. Only patients whose CV's were lower than those of the 30 controls were selected for this study. Electromyographic measurements of anterior tibial muscle potentials and of finger flexor potentials, both at rest and at maximal contraction, showed significant alterations. The patients studied were divided into two groups, depending on the number and severity of clinical signs of carbon disulfide poisoning. Group I patients had both subjective and objective indications of sensorimotor polyneuritis. The predominant subjective symptoms were asthenia, insomnia, psychic depression, and fatigability

while walking; some objective signs were muscular power diminution, "stocking" or "glove" hypoesthesia, and partial or total loss of knee and ankle reflexes. Group II consisted of patients showing only subjective symptoms of disease, primarily lower-limb paresthesia and diminution of muscular power of distal limb muscles.

The author [57] reported that patients of Group I showed considerable slowing of CV's, ankle reflex diminution and abolition, and amyotrophy; they continued to exhibit these symptoms even 1 year after the last exposure to carbon disulfide. In contrast, Group II patients recovered quickly from their reported symptoms. The authors concluded that incipient toxic neuropathy was rapidly reversible, while more serious lesions had poorer prognoses. Neural alterations were first apparent in EMG analysis, and then were reflected by slowing of muscular conduction velocity; hence, EMG's appear to be useful in the diagnosis of early, and probably reversible, symptoms of carbon disulfide neuropathy. The author may have found abnormal alterations in his tests because of the selection of subjects with abnormal CV's.

(d) Effects on the Eyes

Raitta et al [65], in 1974, used neuroophthalmic examinations to study the effects of chronic exposure to carbon disulfide in 100 male viscose rayon workers and 97 male controls. The viscose rayon workers who were part of a previous study [8], had been exposed to carbon disulfide plus hydrogen sulfide at combined concentrations of 10-30 ppm since 1960, 20-40 ppm between 1950 and 1960, and higher than 40 ppm before 1950. Carbon disulfide and hydrogen sulfide had been present in a ratio of approximately 10:1. The mean length of exposure was 15 years; however, 50

of the 100 exposed subjects had been removed from carbon disulfide exposure for reasons of health, transfer, or retirement. For these 50 men, the mean length of absence from exposure was 6 years. The authors reported that 68 of 100 exposed workers and 38 of 97 controls showed delayed peripapillary filling of the choroid ( $P < 0.01$ ). The widths of eight arterioles and of the narrowest vein were found to be significantly greater in the exposed group than in the controls ( $P < 0.01$  and  $P < 0.01$ , respectively). These conditions were thought to have been caused by hemodynamic changes which led to slowed circulation in chronically exposed workers. No evidence indicating a causal role of carbon disulfide in the development of retinopathy (ie, high frequency of retinal microaneurysm) was found.

Raitta and Tolonen [66], in 1975, performed oculophygmography (OSG) and electrocardiography (ECG) simultaneously on 38 male viscose rayon workers exposed to carbon disulfide and 40 nonexposed male papermill workers. The subjects and controls had been found normal in an earlier study [65] when examined for refraction, intraocular pressure, scleral rigidity, and cardiac arrhythmia. The mean age of the exposed group was 51 years, and that of the controls was 49 years. Eighteen of the exposed workers, who had an average of 19 years' exposure, were no longer exposed to carbon disulfide. The controls had been working in the papermill for an average of 4 years. OSG's and ECG's were performed, and intraocular pressures, blood pressures, and pulse rates were measured. No differences were found between the exposed and control groups in these measurements. The authors [66], using ocular wave and ECG information, developed evidence that the ocular vascular beds of viscose rayon workers were more rigid than those of the controls. Those of workers no longer exposed to carbon



disulfide were even more rigid than the vascular beds of currently exposed workers. Drawing on the results of an earlier study [65] in which the microcirculation of the eye was found to be affected early in chronic carbon disulfide intoxication, Raitta and Tolonen [66] concluded that OSG and ECG should both be used as aids in detecting ocular effects attributable to exposure to carbon disulfide.

Szymankowa [67] examined 500 synthetic-fiber workers who had been exposed to carbon disulfide at concentrations reportedly not exceeding 0.01 mg/liter (3 ppm); concentrations of hydrogen sulfide were not reported. The workers were 18-60 years old and had been exposed for periods of 0.5-30 years. Workers exposed for short periods of time (usually less than 5 years) generally had mild visual disturbances such as conjunctival inflammations, temporary corneal opacities, and disturbed color-vision. Prolonged exposure to carbon disulfide was reported to have caused irreversible vascular effects and inflammatory degenerative changes in the retina. In terms of "visual aging" (eg, farsightedness, double vision, accommodation disorders), a chi-square test on 75 carbon disulfide-exposed workers versus 75 controls (plant administrative workers), using the author's data, shows a highly significant increased prevalence of these symptoms in the exposed group ( $P < 0.001$ ). The evidence presented by the author showed a variety of ophthalmic disorders from exposure to carbon disulfide at levels below 3 ppm. However, the lack of a control group to show significance of all findings (rather than just of "visual aging") and the imprecise reporting of carbon disulfide air concentrations and methods of sampling and analysis leave doubts about the validity of the findings.

Maugeri et al [68] measured ophthalmic pressure in 107 viscose rayon workers and 16 unexposed controls. Of the viscose rayon workers, 28 were disabled due to carbon disulfide poisoning, 41 had been exposed for up to 5 years, and 38 had been exposed for up to 10 years. The workers were young; the mean ages were 36 years for the disabled workers, 31 years for workers exposed less than 5 years, 33 years for those exposed 5 years or more, and 31 years for controls. The subjects were homogeneous with respect to birthplace and to living and eating habits. The workplace carbon disulfide concentrations were generally between 200 and 500 mg/cu m (64-161 ppm), with occasional peaks to 900 mg/cu m (289 ppm).

Each subject was given a complete medical examination, with emphasis on visual function. Workers with abnormally high intraocular pressure were excluded from the study. Ophthalmodynamography was then performed on the left eye, and arterial blood pressure was measured in the left arm of each subject.

The authors [68] found that mean systolic and diastolic ophthalmic pressures were significantly higher in the exposed and disabled groups than in the controls. Systemic systolic pressures were approximately 138 mmHg for currently exposed workers and 137 mmHg for the disabled workers versus 115 mmHg for controls ( $P < 0.001$  for both groups). Diastolic pressures were approximately 110 mmHg for currently exposed workers and 113 mmHg for disabled workers versus 87 mmHg for controls ( $P < 0.001$  for both groups). The humoral/ophthalmic pressure ratio was also studied. It is assumed that humoral pressure refers to brachial blood pressure. In the control group, the humoral/ophthalmic systolic ratio was 1.03, while for the currently exposed group it was 0.89 and for the disabled workers, 0.87. For both

disabled versus controls and exposed versus controls, the differences were significant ( $P < 0.001$  in both cases). The humoral/ophthalmic ratios for diastolic pressures were also both significant at  $P < 0.001$ , with values of 0.92 for the controls, 0.73 for currently exposed workers, and 0.71 for disabled workers. The authors [68] stated that low ratios, ie, below those of controls, indicated the existence of carbon disulfide-induced vascular damage of the eye. There were no significant differences in humoral systolic or diastolic blood pressures, although the exposed and disabled groups had slightly higher values than the controls. The authors found that although removal from exposure led to normal humoral blood pressure readings for the workers, the pressure within the ophthalmic artery did not return to normal.

Maugeri et al [68] concluded that peripheral circulatory disturbances caused by carbon disulfide exposure were reversible, while cerebral circulatory disturbances were not. The use of ophthalmodynamography was suggested as a diagnostic test for early signs of carbon disulfide-induced cerebral involvement.

Savic [69] examined 185 viscose workers, most of whom had worked 5-6 years and were between 25 and 35 years old. Of these, 115 worked in the cellulose-fiber operation and had been exposed to carbon disulfide at concentrations of approximately 300 mg/cu m (96 ppm), with peaks occasionally reaching 1,000 mg/cu m (321 ppm). The 70 viscose rayon production workers had been exposed at average concentrations always over 62 mg/cu m (20 ppm) with maximum levels of 176 mg/cu m (56 ppm). "Eye burning" was a complaint of 43.5% of the cellulose-fiber workers and 95.7% of the viscose rayon production workers. Photophobia, "seeing of colors,"

dim vision, and weak night vision were reported by some workers in this group. The authors noted that these effects may have been caused by local irritation of the eyes by hydrogen sulfide, sulfuric acid, and other irritant gases and vapors, as well as by carbon disulfide. Pupillary light reaction was slow, subnormal, and sometimes unequal in 13.9% of the cellulose-fiber workers and in 7.1% of the viscose rayon workers. No differences between the exposed group and a control group of workers of similar age were found on examination of the fundus, of color vision, and of retinal arterial pressure. Methods of examination, criteria for diagnoses, and the selection and use of a control group were not clearly described.

In 1967, Goto and Hotta [70] studied the effects of long-term exposure to carbon disulfide on 1,032 workers from 19 Japanese viscose rayon plants, including 270 unexposed controls. The carbon disulfide concentrations to which the workers were exposed were not reported. The average length of employment for the entire group was between 14 and 15 years. There were no significant differences in age distribution between the controls and carbon disulfide-exposed workers, nor were there any major differences in nutritional or environmental conditions. The workers were examined for a multitude of symptoms and conditions. Urinalyses, blood tests, ECG's, ophthalmoscopy and fundus photography, and neurofunction tests were performed, and the workers were questioned to determine personal history and subjective symptoms.

On the basis of phenolsulfonphthalein and sodium thiosulfate excretion tests, it was found that impairment of kidney function increased with prolongation of exposure to carbon disulfide [70]. Signs of

nephropathy were present in 2.5% of the controls, 3% of the group exposed for less than 10 years, and 9.6% of those with more than 10 years of exposure. No marked differences in blood pressure were noted. The most noteworthy finding by the authors [70] was the greater incidence of retinal microaneurysm in the carbon disulfide-exposed workers. Fundus photography identified 60 cases in 757 exposed workers and 4 in 269 controls (7.9% versus 1.5%;  $P < 0.001$ ). Direct ophthalmoscopic tests revealed 44 cases in 338 exposed workers and only 1 in 121 controls (13.0% versus 0.8%;  $P < 0.001$ ). The authors [70] suggested that there was a trend toward increased numbers of microaneurysms with longer exposures. None of the 41 workers exposed to carbon disulfide for less than 6 years had microaneurysms; however, 7 of 99 (7%) with 6-10 years' exposure, 30 of 219 (14%) with 10-15 years' exposure, and 14 of 118 (12%) with over 15 years' exposure had evidence of retinal microaneurysms. No significant differences were found between the exposed group and the controls in the concentrations in blood of lipoproteins or cholesterol.

Goto et al [71], in 1971, studied retinal microangiopathy by examining a cohort of 214 viscose rayon workers and 45 controls not exposed to carbon disulfide. The authors followed this cohort in subsequent studies. All subjects were men, and the mean ages of the exposed and control groups were similar (32.3 and 33.2 years, respectively). The mean exposure duration of the workers exposed to carbon disulfide was 14.1 years. Prednisolone-augmented glucose tolerance tests (GTT's) were performed on all subjects, their blood being sampled at 1 and 2 hours after glucose ingestion. Although the fasting blood glucose levels of the exposed workers and the controls were very similar, the mean blood glucose

levels of exposed workers at the 1- and 2-hour determinations were significantly higher than those of the controls. The glucose tolerance was found to decrease with longer exposure to carbon disulfide. In the 1-hour prednisolone-GTT, glucose levels were significantly higher in workers exposed to carbon disulfide for 20 years or more than in those exposed for less than 9 years ( $P < 0.01$ ). Blood glucose levels 2 hours after glucose ingestion were also significantly higher in the groups exposed for 10- to 19-years and for 20 or more years than in the group exposed for less than 10 years ( $P < 0.05$ ). Also, there were significant differences between age groups within the exposed cohort and between age groups of exposed workers versus controls. The blood glucose levels were higher in older exposed workers than in younger ones, and exposed workers of age groups 30-39 and 40-49 had higher levels than their corresponding controls.

Goto and associates [71] also performed fundus angiography on 195 exposed workers and 39 controls. Retinal microaneurysms were present in 55.9% of the exposed group and in 15.4% of the controls. The duration of exposure significantly influenced the prevalence of microaneurysms. Of workers with less than 10 years of exposure, 21.2% had microaneurysms, compared with 61.3% of those with 10-19 years of exposure and 75% of those with more than 20 years of exposure. No microaneurysms were found in workers exposed for less than 5 years. Prevalence differed significantly between the less-than-10-year and the 10- to 19-year exposure groups ( $P < 0.01$ ). There was also a significant difference between the group with less than 10 years' exposure and that exposed for 20 or more years ( $P < 0.01$ ).

The microaneurysms were graded by severity according to the following criteria: Grade I--fluorescein angiograph with one or two microaneurysms on venular side of the capillary bed; Grade II--fluorescein angiograph with several microaneurysms not only on the venular side, but also on the terminal arteriolar side of the capillary bed; and Grade III--fluorescein angiograph with numerous microaneurysms of various diameters anywhere in the capillary bed, sometimes complicated with soft exudation or dot hemorrhages. In the exposed cohort, 21.5% had microaneurysms of Grade I, 23.5% of Grade II, and 10.8% of Grade III. In the control group, 12.8% had Grade I, 2.6% had Grade II, and none had Grade III. There were no Grade III microaneurysms in workers with less than 10 years' exposure. Mean blood sugar levels during prednisolone-GTT's in carbon disulfide workers with Grade III microaneurysms were significantly higher than in controls at the 1- and 2-hour determinations ( $P < 0.05$  and  $P < 0.01$ , respectively). The authors [71] hypothesized that carbon disulfide caused a carbohydrate metabolic defect leading to higher blood sugar levels, which paralleled the development of retinal microaneurysms. Further, they suggested that factors responsible for inducing microvascular changes in diabetes mellitus may also cause retinal microaneurysms in carbon disulfide workers, although they did not elaborate. The data presented do not establish conclusively that the altered metabolism of glucose and the production of retinal microaneurysms are related. Carbon disulfide exposure concentrations were not reported.

Goto et al [72], in 1972, studied retinal microaneurysms in carbon disulfide-exposed Yugoslavian viscose rayon workers. By using fluorescein angiography of the fundus of the eye, the researchers again found a greater

frequency of microaneurysms in exposed workers than in controls: 30 of 103 (29.1%) versus 1 of 9 (11.1%). The frequency of microaneurysms increased with duration of exposure. Two of 20 workers (10%) exposed less than 5 years had microaneurysms, as did 5 of 18 workers (27.8%) exposed for 5-8 years, and 23 of 65 (35.4%) exposed for 9-13 years. Five cases of Grade III microaneurysms were found, all in workers with 9 or more years of exposure. Four of these cases were in cellulose-fiber department workers and the other in a spinning department worker. The exposure level was believed to have been higher for the cellulose-fiber workers; however, exposure concentrations were not reported. No significant differences between the exposed and control groups were found in the prednisolone-augmented GTT's or in blood pressure levels. The authors [72] stated that retinal microaneurysm was a main sign in early chronic carbon disulfide intoxication. Also, because these aneurysms were very similar to those seen in early stages of diabetes mellitus and glomerulosclerosis, it was thought that disturbances of carbohydrate metabolism might be involved in carbon disulfide poisoning.

Hotta et al [73] studied the retinal effects of carbon disulfide exposure in 289 exposed workers and 49 unexposed controls in a Japanese viscose rayon plant in 1972. All employees selected were men who resided in the same rural district, had no family history of diabetes, and had no known exposure to other organic solvents. The exposed workers were grouped by level of exposure. The high-exposure group was composed of 124 men who had worked in the spinning or desulfurizing departments or both, and the low-exposure group consisted of 127 men who had worked only in the xanthation, solution, or ripening departments. The remaining 38 exposed



workers had spent time in both high- and low-exposure departments. No exposure concentrations were reported. The mean ages of the exposed and control groups did not differ markedly (42.1 versus 43.3 years). Workers in the high-exposure group had a mean age of 41.4 years and a mean length of exposure of 9.2 years, while workers in the low-exposure group had a mean age of 42.6 years and a mean length of exposure of 11.9 years. Direct ophthalmoscopy was performed on both eyes of each subject. Abnormal findings were then photographed in color with a funduscope. The funduscopy findings were categorized into stages of increasing pathology: Stage 0--no abnormal changes in ocular fundus; Stage I--one or two retinal capillary microaneurysms in the macular region; Stage II--several retinal capillary microaneurysms, a few dot or blot retinal hemorrhages, and a few hard retinal exudates in the ocular fundus; Stage III--numerous retinal capillary microaneurysms, several dot or blot retinal hemorrhages, and a few hard or soft retinal exudates in the ocular fundus.

The authors [73] found that 30.8% of the 289 exposed workers showed signs of retinopathy (Stage I, II, or III), while only 4.1% of the 49 controls showed retinopathic symptoms ( $P < 0.001$ ). Retinopathy was found in 11.3% of the 53 workers exposed for less than 5 years, in 18% of the 86 workers exposed for 5-10 years, in 35.4% of the 79 workers exposed for 10-15 years, and in 54.9% of the 71 workers exposed for more than 15 years. Retinopathy was found in 34.6% of the high-level exposure group and 22.8% of the low-exposure group. The frequency of occurrence of retinopathy in both groups increased with exposure time. The development of retinopathy and its relationship to exposure were studied by examining the prevalence rates of the four stages. In the exposed group, prevalence rates were

lower in higher stage retinopathy. Retinopathy of Stages II and III was not present in controls, but only in workers exposed for more than 5 years. More serious conditions (ie, retinopathic conditions beyond Stage III) were not seen in this study. The authors [73] suggested that this was because of the factory's compulsory retirement age, which would tend to exclude workers with extremely long exposure times. The methods of selection of the subjects and controls were not described in detail. However, assuming that the factory workers were chosen without regard to existing retinopathic conditions, the results are suggestive of a role of carbon disulfide in the development of retinopathy.

(e) Other Effects

Tolonen [74], in 1974, examined 97 male viscose rayon workers and 96 male controls for subclinical symptoms of carbon disulfide poisoning. Using the subjects and controls who had participated in previous epidemiologic studies [8,43,44], Tolonen [74] attempted to identify occupationally poisoned workers based on combinations of signs from cardiovascular, neurophysiologic, ophthalmic, and behavioral examinations. Subjects were classified as having coronary heart disease if they met one or more of the following criteria: (1) verified clinical myocardial infarction, (2) reported myocardial infarction, (3) typical angina, or (4) one or more of the six Minnesota ECG codes. Ophthalmic disturbances were determined by the presence or absence of delayed peripapillary filling, as determined by fluorescein angiography. Polyneuropathy was considered present when reduced CV's were found in two to eight peripheral nerves. Behavioral disorders were considered to exist when the subjects scored poorly on at least two of the psychologic and behavioral tests

administered, which were described by Hanninen [63].

Tolonen [74] found that the relative risks of exposed subjects versus controls ranged from 1.5 to 2.1 for the four categories of disorders. For coronary heart disease, 29 exposed workers were affected versus 19 controls (the difference was not statistically significant,  $P=0.14$ ); for polyneuropathy, 49 versus 23 ( $P<0.01$ ); for disturbed ocular microcirculation, 67 versus 38 ( $P<0.01$ ); and for behavioral deterioration, 39 versus 24 ( $P<0.05$ ). Five exposed workers and 31 controls were free of any disease, while 6 exposed workers and no controls had disorders of all four categories. The results did not indicate any single symptom which could be shown conclusively to be a subclinical manifestation of chronic carbon disulfide poisoning. However, the syndromes (combinations of symptoms) with excess prevalence over controls all included disturbed ocular microcirculation combined with either polyneuropathy, behavioral deterioration, or both. For a particular disorder or syndrome, the probability that the condition was of occupational origin was expressed as its excess occurrence in exposed workers compared with that in controls over its total occurrence in the two groups. Accordingly, the probability that the syndrome that included all four disorders was occupational in origin was 100%, as was the syndrome consisting of cardiac, ocular, and polyneuropathic symptoms (6 versus 0 for both conditions). The author concluded that delayed peripapillary filling was the first indication of chronic carbon disulfide poisoning, followed by polyneuropathy or behavioral deterioration, and finally by coronary heart disease. The sensitive methods used to detect disorders left but a small percentage of subjects symptomless (5% of exposed and 32% of controls). This apparently

low degree of specificity (large number of false positives) makes the individual tests used by Tolonen relatively nondiscriminatory of carbon disulfide intoxication. Conversely, some syndromes occur more frequently with occupational exposure. While this report is of value in attempting to find subclinical signs of carbon disulfide, further work is needed if the tests are to be specific and sensitive enough to screen workers for possible poisoning.

Gondzik et al [10] examined 350 artificial-fiber plant workers to determine if there were changes in the oral cavity associated with exposure to carbon disulfide. The workers had been exposed to carbon disulfide at concentrations of 0.02-0.065 mg/liter (6-21 ppm) and to hydrogen sulfide at 0.002-0.006 mg/liter (1-4 ppm) during the preceding 6 years. A control group of 100 employees from a furniture factory in which there was no exposure to harmful chemical compounds was also studied. The subjects in each group were classified by length of employment. No significant group differences in the prevalence of dental caries were found. The group exposed to carbon disulfide for less than 5 years had significantly lower pH values for both the mucous membrane and the saliva than did the controls (5.28 versus 6.09 and 5.30 versus 6.29, respectively). Workers exposed for longer periods did not show this difference. Based on an index of periodontic disturbances, the authors [10] observed that the frequency of pathologic changes in the periodontium of the exposed workers was significantly higher than that of the controls. The intensity of these changes increased with length of exposure, although the levels of significance did not. The relationship between the observed effects and actual harm to workers was not discussed, and the reliability and validity

of the methods used in detecting these abnormalities were not described.

Kashin [75] studied the effects of carbon disulfide on immunobiologic reactivity and temporary disability in 630 exposed synthetic-fiber factory workers. In the exposed group, 391 workers had been exposed to carbon disulfide at reported concentrations of 30-50 mg/cu m (10-16 ppm), and 239 had been exposed at levels below 10 mg/cu m (3 ppm). The ages of the two exposed groups and the control group of 334 unexposed workers were similar (approximately 90% of each group were less than 40 years old). There were equal numbers of men and women in the two exposed groups; however, 87% of the workers in the control group were women. Immunobiologic reactivity was measured by the Joffe test [76]. This test consists of intradermal injection of a small dose of serum from rabbits immunized to human protein, with grading of the local reaction by diameter and deepness of color of the flare. Negative reaction to the test (ie, reduced immunologic reactivity) was found in 33.2% of the highly exposed group, 25.5% of the moderately exposed group, and 11.4% of the controls [75]. The differences between the two exposed groups and the controls were reported to be significant, although the statistical methods used were not clear. The authors reported a trend toward decreasing reactivity with longer exposure in the highly exposed group. This trend also was found in the moderately exposed group, although it was not evident in those exposed less than 3-4 years. Morbidity involving temporary loss of working capacity was significantly higher in the highly exposed group than in the controls and was also higher in the moderately exposed group, although not significantly. Morbidity was also found to be higher in workers who showed decreased immunologic reactivity by the Joffe test. In all three groups, the workers with

negative or questionable Joffe reactions lost more days of work than those with positive ones, who in turn, lost 1.6-2.0 times as many days of work as those with marked Joffe reactions. This reported relationship between measured reactivity and temporary disability suggests that the Joffe test may be a valid index of harmful effects of exposure to carbon disulfide.

In 1972, Mancuso and Locke [77] conducted the first longitudinal study of viscose rayon workers in the United States. The authors attempted to correlate long-term carbon disulfide exposure with behavioral and mental problems. Suicide was chosen as the indicator of these problems, although the authors recognized its inadequacies (eg, possible underestimation in death-certificate coding, insurance incentives for disguising suicide as the cause of death, variations in exhaustiveness of investigations). The cohort was selected from the personnel records of a viscose rayon plant. For each year between 1938 and 1948, all new employees for whom essential personal and employment data were available were selected for study. From mortality data obtained from the Social Security Administration, the authors located and obtained copies of death certificates of members of the cohort. All death certificates were recoded for cause of death by an experienced nosologist. Person-years of observation were calculated for the period 1938-1968 by age (25-64) and sex. The proportional mortality rates were calculated by dividing the number of deaths in each age group by the person-years of observation for that age group. Multiple job changes by members of the cohort created a problem in analyzing the data. "First job" (meaning first department and occupation in company) was used as a convenient, but not completely valid, method of characterizing and analyzing the work force. Because the number of nonwhites in the plant was

very small, the analyses were confined to a total of 4,899 white employees (3,229 men and 1,670 women).

The authors [77] found the death rate from all causes for the male worker cohort to be below the rate for white male Americans. Similarly, women in the cohort showed a lower than expected death rate from all causes. This coincides with the generally accepted concept of a healthy industrial population. However, the age-adjusted suicide rates (suicides/100,000 persons) for the combined male-female cohort for ages 25-64 was 22.7 (44 suicides) against the corresponding 1955 US rate of 15.1. This difference was significant at the 5% level. In each 10-year age interval between 25 and 64, male rayon workers showed suicide rates higher than the US male population. The 46 reported suicides were then analyzed by department, age, and occupation. However, the small numbers found in most tabular cells do not readily lend themselves to statistical evaluation. There are also numerous other factors besides carbon disulfide that possibly contributed to these suicides. Although the authors attempted to find other correlates with exposure to carbon disulfide, the overall suicide rate was the only variable that could be related to the exposure.

#### Animal Toxicity

The mechanism of action of carbon disulfide and biologic effects that cannot be readily demonstrated in humans have been studied by means of animal experiments.

Seppalainen and Linnoila [78] exposed rats to carbon disulfide to study the development of neuropathy. Forty-six 3-month-old albino Sprague-

Dawley rats, 33 males and 13 females, were divided into 4 study groups. Two groups were exposed to airborne carbon disulfide at 750 ppm (2,330 mg/cu m) in an airtight chamber. One group of 12 rats was exposed 6 hours/day, 5 days/week, for 10 weeks, then 3 days/week for 12 weeks. Four of these rats were observed for a recovery period of 12 weeks after exposure. A group of 15 rats was exposed for 6 hours/day, 5 days/week, for 2-5 weeks, followed by a 12-week recovery period for 5 of the rats. Weight gain and maximal conduction velocities (MCV's) in motor nerves were measured, and the rats were observed for abnormal behavior and clinical signs of neuropathy. A control group of 9 rats was used to measure weight gain; another control group of 10 rats was used to compare the effects of aging and to measure "control" MCV's. Neither control group was exposed to carbon disulfide.

Seppalainen and Linnoila [78] found that the rats exposed to carbon disulfide for 22 weeks were lethargic after each day's exposure and showed some loss of motor activity toward the end of each week of exposure. Recovery occurred quickly during the nightly and weekend rest periods. Clumsiness began at 3 weeks, followed by ataxia at week 6 and weakening of the hindlegs and marked ataxia at 8 weeks. The hindleg condition improved greatly during the 12-week recovery period, but the rats were not so agile as they were before exposure. MCV's decreased steadily during exposure, and, after the first 4 weeks, the MCV's were significantly lower than preexposure levels. Some improvement was seen by the end of the recovery period. The mean weight of the rats remained fairly constant throughout the exposure period and increased during recovery. However, the control rats gained weight more consistently and had greater body weights at the



end of the experiment than did the exposed rats. The rats subjected to short-term exposure (2-5 weeks) to carbon disulfide did not show signs of persistent neuropathy but were lethargic. The MCV's decreased but were quickly reversed after termination of exposure. The MCV's of control rats used to study age effects increased until about 5 months of age but remained fairly constant thereafter. Seppalainen and Linnoila [78] concluded that the finding of decreased MCV's in rats agreed well with the results observed in humans in other studies conducted by Seppalainen and coworkers [55,56], although the changes in humans were less marked.

Szendzikowski et al [79] studied neurohistologic changes in rats chronically exposed to carbon disulfide. Eighty Wistar rats were exposed to carbon disulfide vapor at a mean concentration of 1.5 mg/liter (482 ppm) for 5 hours/day, 6 days/week; 60 rats served as controls. Rats were killed for study monthly from the 1st to the 15th month by one of two methods: total body perfusion with formalin under light anesthesia followed by neural excision (60 rats) or decapitation followed by neural excision and formalin immersion (80 rats). Microscopic examination was restricted to the CNS, including neuronal somas, myelinated fibers, and blood vessels, and the peripheral nerves. The peripheral nerves were embedded in either paraffin or Epon 812, an epoxide resin that allows cutting of thin (1-2  $\mu$ m) sections.

No deterioration of the rats' general condition was noted in the first 7 months of exposure [79]. Thereafter, weight loss, muscular weakness, and loss of motor equilibrium began to appear, followed by physical deterioration, paralysis, and "muscular wasting." Defects in neurons were common in rat tissues prepared by formalin immersion, but rare

in those fixed by formalin perfusion. The authors [79] suggested, therefore, that most of the histologic changes may have been artifactual, ie, based on the method of tissue fixation rather than induced by carbon disulfide exposure. There were no signs of damage to myelinated fibers of cerebrum, cerebellum, or pons. However, the myelinated fibers of the spinal cord of exposed rats showed signs of degeneration, with lesions caused by swelling and disruption of the axons. This axonal swelling became evident as early as 1 month after commencement of exposure. The lesions were distributed symmetrically and involved the ventral and lateral spinal funiculi. The blood vessel walls of exposed rats did not differ in appearance from those of controls. The routine paraffin technique revealed progressive degeneration in the myelinated fibers of peripheral nerves similar to that seen in the spinal fibers. The histologic findings in paraffin sections, although abnormal, were not in proportion to the advanced functional impairment found in rats exposed to carbon disulfide for long periods. A number of pathologic alterations were found in neuronal sections embedded in Epon 812. The major changes observed included variations in fiber diameters, total breakdown of individual fibers, structural changes in neuron fibers (eg, shrinkage, disruption, and loss of axons), and an increased amount of interstitial tissue. Szendzikowski et al [79] found that all typical morphologic changes could be demonstrated by the 5th or 6th month of exposure, before the appearance of functional changes, using paraffin sections. The use of Epon sections allowed detection of structural changes in the peripheral nerves much earlier (after 1 or 2 months).

Gondzik [80], in 1971, reported the results of studies of the effects of carbon disulfide on testicular tissues of rats. Three experiments were conducted using 85 mongrel rats, 2-5 months old and weighing 200-260 g. In the first experiment, 12 rats were injected ip every 2nd day for 60 days with 12.5 mg/kg of distilled carbon disulfide dissolved in peanut oil; 5 were given pure peanut oil; and 5 were untreated. In the second experiment, 15 animals were given ip doses of 25.0 mg/kg every other day for 60 days; 10 rats were given pure peanut oil; and 9 were untreated. In the third experiment, 10 were given 25.0 mg/kg ip every other day for 120 days; 10 were injected with peanut oil; and 9 were untreated. After each experiment, the animals were decapitated and autopsied. The testicles were fixed in formalin and cut perpendicularly to the long axis at both poles and at the point of greatest diameter. A total of 1,020 sections were prepared and analyzed from the 170 gonads examined.

The testicles of rats from exposed and control groups had similar histologic and histochemical patterns. However, exposed rats had thickened vascular walls, blood-cell-engorged vessels, disorganized seminiferous epithelium, and decreased numbers of spermatozoa. Rats injected with carbon disulfide for a 120-day period, however, showed marked testicular damage. Advanced regressive lesions involving all parts of the testicles were found. The most pronounced changes were the "folding and shrinkage" of the usually round and smooth tubular basement membrane. Spermatogonia were few and sometimes nonexistent in the seminiferous tubules, and spermatogenesis was absent. Leydig cells showed degeneration and atrophy. Gondzik [80] concluded that carbon disulfide caused irreversible microscopic disturbances in testicular structure, but he did not quantify

the results of the tests in terms of numbers of animals showing abnormalities and of the significance of differences.

Yaroslavskiy [81] exposed female albino rats and mice to carbon disulfide vapor to study its effects on the course and duration of pregnancy. The animals, in groups of 12-20, were exposed to carbon disulfide at a concentration of 2,000 mg/cu m (642 ppm) for 2 hours/day during the entire pregnancy. In some experiments, mice were given 200-mg/kg doses of tryptophan instead of, or in addition to, carbon disulfide. Two identical series of tests were performed on rats. In the first experiment, 16.8% preimplantation embryonic mortality occurred in the 12 exposed animals and 3.3% in the 12 controls ( $P < 0.05$ ). In the second experiment, the preimplantation mortality rate was 22.6% in 12 exposed rats and 6.5% in 14 controls ( $P < 0.05$ ). The reproductive success of each exposed group was lower than that of its control group in both experiments (6.8 versus 9.7 fetuses per rat,  $P < 0.05$ , and 8.0 versus 9.3 fetuses per rat). There were seven postimplantation deaths in the fetuses of exposed rats and none in those of the controls. There were no significant differences between experimental and control rats in the mean corpus luteum counts or in mean fetal weights.

In mice, the preimplantation fetal mortality rate was 11.3% for 21 controls, 18.8% for 15 carbon disulfide-exposed mice (group A), 27.7% for 20 mice exposed to carbon disulfide plus tryptophan (group B), and 12.6% for the 20 mice exposed to tryptophan only (group C). Differences from the control group were significant for the mice exposed to carbon disulfide alone ( $P < 0.001$ ) and to carbon disulfide plus tryptophan ( $P < 0.05$ ). The mean number of live fetuses per mouse was 8.6 in controls, 6.6 in group A

( $P < 0.05$ ), 6.3 in group B ( $P < 0.05$ ), and 8.1 in group C (not significant). No embryos of control mice died after implantation, but groups A, B, and C had 8, 3, and 5 embryo deaths, respectively.

Yaroslavskiy [81] concluded that carbon disulfide slightly affected the reproductive success of the animals but had no effect on the weights of the newborn rats. Embryo toxicity was found in the exposed animals during the preimplantation and postimplantation periods, however, no terata were found in the litters from the dams exposed to carbon disulfide. Tryptophan and carbon disulfide were found to act synergistically as fetotoxic compounds. Because carbon disulfide and its metabolites have been said to lead to increased serotonin concentrations in the blood and brain and tryptophan is a precursor of serotonin, the author [81] believed that his results indicated a blockage of serotonin biotransformation.

Petrun [82] studied the biochemical effects of dermal exposure of rabbits to carbon disulfide. The shaved backs of rabbits of unspecified sex were exposed to carbon disulfide vapor for 2 hours while the animals breathed uncontaminated air. Fourteen rabbits were exposed to carbon disulfide at 2 mg/liter (642 ppm), and 14 were exposed at 10 mg/liter (3,210 ppm). Both before and after exposure, the rabbits were studied for magnitude of pulmonary gas exchange, gaseous composition of venous blood, hemoglobin level, erythrocyte count, rate of aerobic and anaerobic glycolysis of erythrocytes, protein fractions of blood serum, carbonic anhydrase activity, and blood cholinesterase activity. Statistically significant changes found in the exposed animals were increased carbonic anhydrase activity, decreased anaerobic glycolysis of erythrocytes, and decreased cholinesterase activity in erythrocytes in both groups, and

increased albumin and decreased globulin in the group exposed at 2 mg/liter (642 ppm). In addition, the percentage of carbon dioxide in the venous blood increased from 31.3% to 35.8% ( $P < 0.01$ ) in the more highly exposed group. Petrun [82] concluded that these brief exposures to carbon disulfide caused harmful effects in rabbits, even though there were no visible signs of intoxication. While the author measured several significant differences in biochemical parameters in rabbits, he did not clearly explain their importance and ramifications. Changes in blood characteristics after exposure do not in themselves indicate toxicity, and extrapolation of these animal data to human exposure requires additional evidence.

Cohen et al [83], in 1958, studied skin absorption of carbon disulfide vapor in male albino rabbits. Each animal (total number not specified), with 40% of its fur clipped, was placed in a chamber so that only its body and legs were exposed to carbon disulfide vapor. Uncontaminated air was supplied through a face mask, and exhaled air was collected in a respirometer. Cutaneous absorption of carbon disulfide, reaction of carbon disulfide with free blood amino groups, blood zinc concentrations, and skin histopathology were studied. After 3 hours of dermal exposure to carbon disulfide at 1,550 ppm (4820 mg/cu m), the exhaled air of the rabbit contained 2.5 ppm (7.8 mg/cu m) of the compound; 0.25 ppm (0.78 mg/cu m) could still be detected 1.5 hours after cessation of the 3-hour exposure. Exposure of one rabbit to carbon disulfide at 1,500 ppm (4665 mg/cu m), 3 hours/day for 8 consecutive days revealed that the concentration of carbon disulfide in the exhaled breath increased with the length of exposure. In an experiment with single 70-minute, whole-

body, cutaneous exposures at varying concentrations, a linear increase in the concentration of carbon disulfide exhaled in the breath was found as the exposure concentration was increased. However, no exhaled carbon disulfide could be detected at exposure concentrations of 150 ppm (465 mg/cu m) or less, even after 6 hours of exposure. The ultraviolet absorption spectrum of serum from a rabbit dermally exposed to an unspecified concentration of carbon disulfide for 3 hours/day for 8 consecutive days showed a decreased transmittance between 290 and 325  $\mu$ . The authors suggested that this indicated a reaction between carbon disulfide and amino groups of serum proteins. The concentration of zinc in serum and erythrocytes decreased following dermal exposure to carbon disulfide. No microscopic changes were seen in sections of skin from exposed rabbits. Cohen et al [83] suggested that dermal exposure to carbon disulfide vapor may produce chronic carbon disulfide intoxication, especially with long exposure. Because apparently very few rabbits were used (some graphs showed data from only one animal) and no examination of statistical significance was done, this study should be considered exploratory.

Cohen et al [84], in 1959, studied the biochemical changes due to carbon disulfide in 11 male New Zealand white rabbits. The rabbits were exposed to carbon disulfide by inhalation for 6 hours/day, 5 days/week, for up to 38 weeks. Concentrations of carbon disulfide were 250 ppm (775 mg/cu m) during the first 16 weeks, 500 ppm (1,555 mg/cu m) for the next 5 weeks, and 750 ppm (2,330 mg/cu m) for the final 17 weeks. There were six controls. The concentration of carbon disulfide in the exhaled breath was measured colorimetrically 16 hours after termination of exposure. Blood

samples were taken weekly from all animals. Two exposed and one control animal were killed by pentobarbital injection after 12 weeks and examined; one exposed animal and one control were killed after 28 weeks; four exposed animals and two controls were killed within 1 week after exposure was stopped at week 38; the other four exposed rabbits and two controls were killed after a 6- to 7-week period of "observation and recovery."

The major sign of toxicity was partial but irreversible hindleg paralysis, which followed marked loss of body-weight beginning about week 24 of the exposure [84]. Carbon disulfide in the exhaled breath averaged 1.4 ppm (4.3 mg/cu m) when the exposure concentration was 500 ppm (1,555 mg/cu m) and rose to 3.1 ppm (9.6 mg/cu m) when the exposure concentration was 750 ppm (2,330 mg/cu m). No carbon disulfide was detected in the exhaled breath of rabbits exposed at 250 ppm (775 mg/cu m). Exposed animals did not gain weight during the first 11 weeks of exposure, although the controls did. During the period between weeks 11 and 24, however, the exposed rabbits gained weight steadily, although slightly less rapidly than did the controls. After week 24, the exposed animals began to lose weight. After exposure ceased, at week 38, the animals gained some weight.

The authors [84] found that total serum cholesterol increased in exposed rabbits when the carbon disulfide concentration was increased to 750 ppm (2,330 mg/cu m) and returned to normal after exposure ceased. Hematocrit readings and sedimentation rates in exposed animals did not differ significantly from those in the controls. Electrocardiograms taken on all animals after signs of toxicity appeared showed no abnormalities, and neither T-wave inversion nor R-S-T segment abnormalities were detected. Results of urinalyses, taken irregularly, were unexceptional.



Microscopic findings considered abnormal were centrilobular congestion and mild fatty degeneration of the liver, mild hemosiderosis of the spleen, and chronic interstitial nephritis. Ten exposed rabbits had varying degrees of adrenal hyperplasia; adrenal cortical adenomata were found in not only four of these animals but also two controls. The mean adrenal weight of the exposed rabbits was nearly twice that of controls. The CNS was the site of the most marked pathologic changes. As early as 12 weeks after exposure began, meninges of the brain were swollen, proliferatively thickened, and infiltrated with lymphocytes. Individual nerve cells in the cortices of all exposed animals showed changes that included vacuole formation, cytoplasmic fraying, swollen nuclei, and tortuous dendrites. Cerebellar damage consisted chiefly in decreased numbers of, and degenerative changes in, Purkinje cells. The most striking CNS changes involved the spinal cord. All exposed rabbits showed spinal cord damage in the upper thoracic region. No demyelination was seen, nor was there any optic nerve damage. Cohen et al [84] found increased urinary and fecal excretion of zinc by the exposed rabbits and a gradual decrease in the mean concentration of zinc in the blood serum during the study.

Cohen et al [84] outlined the following theory for the mechanism of carbon disulfide toxicity: inhaled carbon disulfide reacts with amino groups of proteins and amino acids to produce thiocarbamates and thiazolidones. The presence of these substances was suggested by changes in the ultraviolet absorption spectra of sera from exposed rabbits. These proposed metabolites could chelate such metals as zinc, so that enzymes that require activation by these metals would be inhibited by deprivation of their activators. There were also decreased activities of serum and

tissue alkaline phosphatases (magnesium-requiring enzymes). The decreased activities of the alkaline phosphatases of serum and tissues in exposed rabbits were thought to result from chelation of magnesium (a member of Group IIA of the periodic table) similar to that postulated for zinc (a member of Group IIB). When one considers that zinc and other divalent metals (eg, magnesium, cobalt, and copper), all of which would be susceptible to chelation by compounds able to form complexes with zinc, are required by such enzymes as carbonic anhydrase and creatine phosphokinase, among others, it becomes apparent that the postulated chelation of these metals by thiocarbamates and thiazolidones could have substantial effects on cellular metabolism and even integrity. This hypothesis should be considered tentative, since the authors did not specify the length of exposure that preceded the sampling of tissues of the individual rabbits for estimates of zinc concentrations and no controls were used in the zinc excretion studies.

Misiakiewicz et al [85] studied the toxic effects of low doses of carbon disulfide alone and in combination with hydrogen sulfide. Groups of 11 male Wistar albino rats each were exposed in separate inhalation chambers to (1) 0.1 mg/cu m (0.03 ppm) carbon disulfide, (2) 0.1 mg/cu m (0.03 ppm) carbon disulfide plus 0.1 mg/cu m (0.07 ppm) hydrogen sulfide, (3) 1.0 mg/cu m (0.3 ppm) carbon disulfide, or (4) 1.0 mg/cu m (0.3 ppm) carbon disulfide plus 1.0 mg/cu m (0.7 ppm) hydrogen sulfide. A control group of 11 rats was exposed to air alone under the same conditions as the experimental animals. Gas concentrations in the 150-liter chambers were very nearly constant, with an airflow of 30 liters/minute. Exposures were continuous for approximately 160 days except during feeding and the taking

of blood and urine samples. Animals were examined for body weight increases, coproporphyrin concentration in the urine, blood cholinesterase activity, serum aspartate aminotransferase activity, and histopathologic abnormalities. All groups of rats gained weight during the experiment, although experimental animals gained less than the controls. Mean body-weight changes, as percent reduction compared to controls, were 7.5, 15.4, 23.3, and 28.9%, respectively, for the four exposure groups. Weight changes in groups 3 and 4 were significantly different from those of the controls. Urinary coproporphyrin concentrations in groups 2, 3, and 4 increased 14, 57, and 100% from their initial levels, but the level did not increase in group 1. In group 4, the increase was significant at the 5% level.

Statistically significant increases in blood cholinesterase activity were seen in all exposed groups by day 85 of the study. The increases continued until day 153 in all but group 4. Serum aspartate aminotransferase activity rose markedly in groups 2 and 3 and especially in group 4. Aminotransferase activity in this group was 118% higher than in the controls, while groups 1, 2, and 3 showed increases of 14, 64, and 98% over the controls. Microscopic examination revealed no differences in histopathology between group 1 and the controls. Chronic inflammation of the lobular bronchi and bronchogenic inflammation of the lungs were present in rats of groups 2 and 3. Chronic inflammation of the segmented bronchi was the predominant finding in group 4 rats. Misiakiewicz et al [85] concluded that the combination of carbon disulfide and hydrogen sulfide was more harmful than carbon disulfide alone, citing exacerbated effects when the combination was present. Harmful effects on the rats were found at a

carbon disulfide concentration of 0.1 mg/cu m.

There are several weaknesses in this report. With the exception of the body-weight measurements, tests were performed on only 5 or 6 rats in each group, rather than on all 11. This limited number and the lack of statistical measures of variation detract from experimental reliability. The colorimetric method of cholinesterase activity measurement is not noted for reliability, and the biphasic behavior of group 4 with respect to this enzyme is peculiar. Also, the authors could have more precisely evaluated the effect of the combination of carbon disulfide and hydrogen sulfide by including groups exposed only to hydrogen sulfide.

Wakatsuki and Higashikawa [86] studied the toxic effects of carbon disulfide and hydrogen sulfide on nine mature rabbits, divided into three groups of two males and one female. Group 1 was exposed by inhalation to carbon disulfide at 300 ppm (930 mg/cu m), 30 minutes/day for 120 consecutive days. The second group was exposed to hydrogen sulfide at 100 ppm (140 mg/cu m) and the third group was given 300 ppm (930 mg/cu m) of carbon disulfide plus 100 ppm (140 mg/cu m) of hydrogen sulfide on the same exposure schedule. Two untreated rabbits were used as controls. The animals were killed 140 days after exposure ceased, and tissues from hematopoietic organs, excretory organs, heart, lungs, testes, and ovaries were prepared for study.

No significant differences in bone-marrow activity were observed between groups 1 and 2 and the controls. In the mixed-gas group, however, there was a marked increase in bone-marrow cells. All three groups showed mild regeneration of hepatic cells, but group 3 rabbits also showed central fatty degeneration of the liver. Severe hyperemia was seen in the spleens

of rabbits in the the mixed-gas group, but no substantial changes were seen in the other two groups. Very mild kidney changes were seen in groups 1 and 2, but group 3 rabbits exhibited, in addition to the minor change found in groups 1 and 2, degeneration of the epithelium of the uriniferous tubules and presence of calcium deposits. There were no significant testicular changes in the carbon disulfide group; diminished spermatogenic capability resulting from seminiferous tubular atrophy was seen in the hydrogen sulfide group; spermatogenesis ceased entirely in the rabbits exposed to a combination of the gases. No impairment of the lungs, heart, or ovaries was observed in any of the rabbits.

Wakatsuki and Higashikawa [86] concluded that marked histologic changes occurred in rabbits exposed to the mixture of carbon disulfide and hydrogen sulfide, and only insignificant changes resulted from exposure to either gas individually. The authors explained that bone-marrow cell proliferation probably was accelerated in the rabbits of group 3 because they were probably still undergoing recovery in the 140 days after cessation of exposure. The authors also believed that the reasons there were no major abnormalities observed in groups 1 and 2 were that the short daily exposure duration (30 minutes) did not allow adequate time for toxic action, and that the 140 days following the end of exposure and preceding examination was too long and thus allowed recovery. That is, damage done by carbon disulfide or hydrogen sulfide alone was generally reversed after 140 days of recovery, whereas the combination of the vapors produced effects from which the rabbits were unable to recover. Therefore, toxic synergism was present when carbon disulfide and hydrogen sulfide coexisted.

There are several shortcomings in this report. Data are presented qualitatively only, and generalizations based on groups of three animals are unreliable. The authors used only two controls and did not use the data from them in describing changes in the exposed rabbits. Also, the description of experimental procedures fails to explain the researchers' methods clearly.

Wakatsuki [87], in 1959, also reported on the possible toxic synergism of the combination of carbon disulfide and hydrogen sulfide in rabbits. Three groups of four animals each were exposed under the same conditions described by Wakatsuki and Higashikawa [86] and compared with the same number of unexposed controls [87]. The general health, body weight, and blood conditions of the rabbits were examined. Four months after the end of exposure these factors were again examined, and recovery was monitored. Neither carbon disulfide nor hydrogen sulfide alone caused major changes in the rabbits' conditions. The minor changes observed were quickly reversed with no further abnormalities. However, exposure to the combination did cause several changes. Among these were lowered food intake, decreased body-weight gain, decreased hemoglobin and red blood cell count, increased white blood cell count, reticulocytosis, and lowered specific gravity and albumin/globulin ratio of the blood. Wakatsuki [87] found that when comparatively high concentrations of carbon disulfide and hydrogen sulfide are mixed, toxic action is reinforced. The author stated that it would therefore be dangerous to allow carbon disulfide and hydrogen sulfide to coexist at the MAC's intended for human exposure to either gas alone. This report [87] is more detailed than that of Wakatsuki and Higashikawa [86], but the author still used few animals and procedures and

findings are often not clearly described.

Barilyak et al [88] studied the effects of a combination of carbon disulfide and hydrogen sulfide on reproduction in rats. Rats were exposed to carbon disulfide plus hydrogen sulfide at a combined concentration of 10 mg/cu m (the actual individual concentrations of carbon disulfide and hydrogen sulfide were not given). In the first experimental group, 11 females and an unspecified number of males were exposed continuously for 70-90 days and then mated; the pregnant females were then kept exposed under the same experimental conditions until the 20th day of gestation. In the second group, 13 females exposed for 70-90 days were then mated with unexposed males and kept under exposed conditions. In group 3, an unspecified number of males exposed for 70 days were mated with 11 unexposed females, with gestation under control conditions. The 12 females of group 4 were exposed during days 1-20 of gestation. In group 5, 11 females were exposed for 70-90 days, kept under control conditions for 70 days, and then mated to unexposed males. A control group of 26 females were mated to unexposed males. All pregnancies were terminated on day 20 of gestation. Fetuses were examined for terata, and portions of liver and kidneys were taken from both mothers and fetuses for microscopic examination. Numbers of corpora lutea, implantation sites, and live fetuses were determined for each group. These figures were used to calculate the numbers of embryonic deaths before and after implantation and the total number of intrauterine deaths.

The mean numbers of live fetuses per rat were 5.4, 3.8, 6.4, 6.7, 6.5, and 9.0 for groups 1-5 and controls, respectively. The corresponding percentages of deaths of concepti given by the authors were 50, 62, 39, 22,

35, and 9.3%; however, calculation from the authors' data gives a value of 35% rather than 22% for group 4. Carbon disulfide and hydrogen sulfide showed definite embryotoxicity; calculations from the authors' data show that differences between exposed and control rats were significant for all groups. The most pronounced effects were in group 2, although group 1 was also markedly affected. Even when exposed animals were not mated until 70 days after the end of exposure, preimplantation and postimplantation mortality rates were significantly higher than in controls ( $P < 0.005$  and  $P < 0.001$ ). In group 1, there were 2 cases of hydronephrosis in the 32 fetuses, and 1 fetus in 24 had a supernumerary 14th rib. Also, 5.4% of the embryos in group 1 showed "developmental anomalies," whereas there were none in the controls. In group 2, two fetuses had hydrocephaly and hydronephrosis. Microscopic examination showed changes in small blood vessels in the livers of the embryos of groups 1 and 2. Eight rats of group 1 and four of group 2 had retarded ossification. Rats of other groups did not show such substantial abnormalities. Barilyak et al [88] described their data as showing a "weak teratogenic effect" of exposure to carbon disulfide and hydrogen sulfide. However, data and many methodologic details of this study are inadequately reported. It is unclear whether the rats were exposed to 10 mg/cu m of carbon disulfide and 10 mg/cu m of hydrogen sulfide or to a total concentration of 10 mg/cu m. It is difficult to interpret the results quantitatively since individual litter data were not given. Proper statistical tests were not performed on the teratologic data; therefore, conclusions about the teratogenic effects of exposure to carbon disulfide and hydrogen sulfide cannot be made with confidence.



### Correlation of Exposure and Effect

There is an abundance of epidemiologic data on occupational exposure to carbon disulfide, and adverse health effects have been well documented. A summary of workplace exposures and effects are presented in Table III-1. However, the reports cited as evidence of effects on human health from carbon disulfide exposure are all from viscose rayon manufacture and thus include exposure to hydrogen sulfide in addition to carbon disulfide. While investigators have usually obtained measurements of airborne carbon disulfide concentrations, the concurrent concentrations of hydrogen sulfide have rarely been measured. With the exception of the Finnish studies [8,43-47,55,56,63,65,66]. the reports on occupational exposure to carbon disulfide provide little or no data on environmental conditions. The studies generally report mean concentrations without supportive data, possibly not representative of actual workplace exposures.

Studies of animal toxicity have been used sparingly in the document because of the large amount of available data on occupational exposure to carbon disulfide. The animal data primarily corroborate results found in humans or demonstrate biologic effects not demonstrated in man. A summary of results of exposures of animals to carbon disulfide or carbon disulfide plus hydrogen sulfide appears in Table III-2.

Several investigators have studied the cardiovascular effects of exposure to carbon disulfide [8,31,33,41,43-49,51,54]. Hernberg et al [8], in 1967, developed a cohort of 343 viscose rayon workers and 343 controls and prospectively studied their cardiovascular morbidity and mortality. They found higher morbidity and mortality rates from coronary heart disease in viscose rayon workers than in controls. The latest followup of the

cohort [47], in 1976, showed decreased coronary mortality ostensibly resulting from decreased exposure levels. However, this good study presents documented evidence of cardiovascular effects of chronic exposure to carbon disulfide at concentrations of 10-30 ppm (31-93 mg/cu m). Coronary mortality, hypertension, angina, abnormal plasma glucose and creatinine levels, and "coronary ECG's" were all found [8,43-47]. Other studies [41,48] have reported that, compared to controls, there was increased risk of coronary heart disease in viscose rayon workers at concentrations around 20 ppm (62 mg/cu m). Another study [33] found atherosclerosis and hypertension in workers exposed to carbon disulfide at concentrations as low as 7 ppm (22 mg/cu m). These studies document a correlation between cardiovascular problems and occupational exposure to carbon disulfide.

Vascular disturbances involving the eyes were related to carbon disulfide exposure in several reports [65-73]. Among the conditions reported were vascular rigidity, slowed circulation, increased ophthalmic pressure, and retinal microaneurysms. Retinal degeneration and conjunctival inflammation were reported at carbon disulfide concentrations below 3 ppm [67], although methods used to determine the concentrations were not reported. Vascular encephalopathy has been reported after exposure to carbon disulfide at concentrations of 10-482 ppm (31-1,500 mg/cu m) [31]. Reduction of renal plasma flow and renal circulatory ratio and increased total renal resistance were described as manifestations of systemic vascular alterations caused by carbon disulfide [32]. Vascular effects of long-term exposure to carbon disulfide have been manifested in the heart, eyes, kidneys, and brain, and these are probably the most

important documented effects caused by carbon disulfide exposure.

Neuromuscular effects from exposure to carbon disulfide have been demonstrated in several reports [54-57,64], with symptoms including CNS and peripheral nerve damage, abnormal EMG's, slowed conduction velocities (CV's), and muscular weakness. Increased muscular excitability has been reported in 19-year-old women exposed to carbon disulfide at concentrations below 3 ppm (9 mg/cu m) for just 9 months [54], and diminished muscular power and slowed CV's and reflexes were found in young workers exposed at a mean concentration of 5 ppm (15 mg/cu m) [57]. The former study did not report sampling or analytical methods, and the latter reported carbon disulfide concentration peaks of up to 225 ppm (700 mg/cu m), casting doubt on the actual mean concentration experienced by the workers. Muscular weakness, paralysis, and myelin and neuron degeneration were observed in rats exposed at a concentration of carbon disulfide of approximately 482 ppm (1,500 mg/cu m) [79] and slowed maximal conduction velocities (MCV's) and muscular weakness were observed in rats exposed at 750 ppm (2,330 mg/cu m) [78].

Several other health effects related to carbon disulfide exposure have been reported. Among these were psychologic disturbances at 10-40 ppm (31-124 mg/cu m) [63], periodontic changes at 6-22 ppm (19-68 mg/cu m) [10], and immunologic abnormalities at 3-16 ppm (9-50 mg/cu m) [75]. The periodontic changes are of dubious importance and neither the immunologic changes nor the concentrations at which they occur are well established. Tuttle et al [64] reported that a battery of behavioral tests could effectively detect neurologic changes in occupationally exposed subjects prior to the manifestation of clinical symptoms.

Several reports have dealt with possible dermal absorption of carbon disulfide. A human experiment [37] showed that skin absorption of carbon disulfide from aqueous solutions, as commonly used in viscose rayon spinning operations, was substantial, but no dermal absorption of the vapor was noted. Experiments [82,83] studying the effects of prolonged dermal exposure to carbon disulfide vapor on rabbits yielded evidence of absorption.

Metabolic studies in humans [40] and in rabbits [84] were reported, but results were more suggestive than conclusive. The conclusion of the human study [40] was that carbon disulfide concentrations below 20 ppm could retard metabolism of commonly used drugs, allowing the drug to persist in the body longer than normal. The study on rabbits [84] led the authors to develop a theory of the mechanism of carbon disulfide toxicity: Inhaled carbon disulfide reacts with amino groups of proteins and amino acids to produce thiocarbamates and thiazolidones. These compounds chelate metals so that enzymes that require metal activators are thus inhibited. The results are cellular metabolic disturbances and corresponding toxic effects.

Several reports [85-87] described the effects on animals of exposure to carbon disulfide alone, hydrogen sulfide alone, and the combination. These studies concluded that exposure of animals to the combination produced exacerbation or synergism of toxic effects of the compounds. However, several reports [8,9,41] have ascribed the toxic effects demonstrated in viscose rayon employees to carbon disulfide alone. Experimental animal studies [78-81] strengthen this interpretation by demonstrating effects in animals exposed to carbon disulfide similar to

those found in viscose rayon workers. The cases presented on both sides of the synergism argument are weak.

The data on the results of exposure to carbon disulfide present a wide range of significant, adverse effects on health. However, the airborne carbon disulfide concentrations corresponding to these effects are not so reliable nor so consistently reported. In correlating effects with levels of exposure, it is important to emphasize those studies which present environmental monitoring methods and results.

#### Carcinogenicity, Mutagenicity, Teratogenicity, and Effects on Reproduction

No reports on carcinogenesis or mutagenesis resulting from exposure to carbon disulfide or the combination of carbon disulfide and hydrogen sulfide have been found.

Barilyak et al [88] reported a weak teratogenic effect in rats following low-level exposures to a combination of hydrogen sulfide and carbon disulfide. This study does not present strong evidence of teratogenic effects from the mixed exposure, and, without corroborating studies, the results of this investigation must be considered tentative.

Effects on the reproductive system resulting from occupational exposure to carbon disulfide have been demonstrated [58-62]. Women viscose rayon workers exposed at concentrations of less than 3 ppm (9 mg/cu m) had numerous disorders of menstrual and ovarian function [60]. An increased risk of spontaneous abortions at concentrations down to 9 ppm (28 mg/cu m) and below was reported [61,62]. Infertility, threatened pregnancy terminations, and menstrual disorders have also been reported. Young men have experienced spermatic disorders following short-term exposures at concentrations of 13-26 ppm (40-71 mg/cu m) [58,59]. All of these studies

report reproductive system effects after carbon disulfide exposure at concentrations reportedly below the existing OSHA TWA concentration limit of 20 ppm (62 mg/cu m). Other studies [34-36] have not shown such reproductive system disorders, and their authors have concluded that, except at high concentrations, no adverse health effects result from exposure to carbon disulfide. These studies leave unsettled the question of adverse effects by carbon disulfide on human reproductive functions. Findings of reproductive system disorders in women have not been confirmed in this country.

Animal studies have shown adverse reproductive effects following carbon disulfide inhalation. Increased intrauterine mortality and decreased reproductive success [81] at a carbon disulfide concentration of 642 ppm (1,997 mg/cu m) and testicular lesions and cessation of spermatogenesis [80] at 25 ppm (78 mg/cu m) were shown in rats and mice. Increased fetal mortality and questionable teratogenesis were observed in rats exposed to low concentrations of carbon disulfide and hydrogen sulfide (total concentration of 10 mg/cu m) [88].

TABLE III-1

EFFECTS OF OCCUPATIONAL EXPOSURE TO CARBON DISULFIDE  
PLUS HYDROGEN SULFIDE

No. of Workers	Age: Mean or Range	Concentration (mg/cu m)*		Duration: Mean or Range (years)	Effects	Ref- erence
		CS2	H2S			
100	-	450-1,000	-	-	Polyneuritis in 88%, gas- tric disturbances in 28%	31
43	53	30-1,500	-	21	Encephalopathy	31
107	32	200-400	-	1-9	Ophthalmic pressure 138/ 110, vs 115/87 in controls	68
185	25-35	62-174	-	ca 5	Eye burning in 96% of rayon-production workers, 44% of cell-fiber workers; pupillary light reactions abnormal	69
100	39	31-137	-	10	Psychomotor and psycho- logic disturbances	63
125	47	ca 124	-	13	Coronary heart disease in 5.6%, vs 1.2% in controls	48
33	22	40-81	-	2	Asthenospermia, hypo- spermia, teratospermia	58
350	33	19-65	1-6	6-10	Periodontal changes	10
116	50	>62	-	>5	Coronary heart disease in 16.5%, vs 2.7% in controls	49
28	44	ca 62	-	13	Coronary heart disease in 3.6%, vs 1.2% in controls	48
38	51	29-118**	***	20	Ocular vascular rigidity	66
100	48	29-118**	***	15	Ophthalmic circulation slowed	65

TABLE III-1 (CONTINUED)

EFFECTS OF OCCUPATIONAL EXPOSURE TO CARBON DISULFIDE  
PLUS HYDROGEN SULFIDE

No. of Workers	Age: Mean or Range	Concentration (mg/cu m)*		Duration: Mean or Range (years)	Effects	Reference
		CS2	H2S			
118	-	29-118**	***	15	Polyneuropathy, abnormal EEG's	56
343	45	29-118**	***	11	Angina in 17%, vs 11% in controls; blood pressure 140/91, vs 136/85 in controls; coronary heart disease cause of 52% of deaths, vs 31.7% nationally	8
343	45	29-118**	***	11	Fasting glucose levels increased with longer exposures; plasma glucose levels higher than in controls	43
319	45	29-118**	***	>10	Coronary heart disease mortality 5.6 times that in controls; total mortality 2.7 times controls	44
322	45	29-118**	***	>10	Coronary heart disease more frequent than in controls: fatal infarctions 4.8, total infarctions 3.7, nonfatal infarctions 2.8, angina 2.2, "coronary ECG's" 1.4 times higher than controls	45
343	25-72	29-118**	***	>10	Life expectancy decreased 0.9-2.1 years, depending on age, during 8-year followup	47



TABLE III-1 (CONTINUED)

EFFECTS OF OCCUPATIONAL EXPOSURE TO CARBON DISULFIDE  
PLUS HYDROGEN SULFIDE

No. of Workers	Age: Mean or Range	Concentration (mg/cu m)*		Duration: Mean or Range (years)	Effects	Reference
		CS2	H2S			
36	42	29-118**	***	>6	Peripheral nerve and CNS damage; conduction velocities slowed; EMG's abnormal	55
397	35-64	29-118**	***	-	Coronary heart disease cause of 42% of deaths in highly exposed workers, 24% in moderately exposed, 14% nationally	41
165	53	<59**	***	<10	Coronary mortality during 8-year followup 5.8%, vs 2.6% in controls; total mortality 10.2% vs 6.7% in controls	17
630	20-40	31-50	-	-	Immunologic reactions decreased; job absenteeism increased	75
138	<50	22-44	-	<10	Arteriosclerotic changes in 30.4%, hypertension in 23.2%	33
94	18	12-31	<10	<1	Hypotension, nervous system excitability	54
189	<30	ca 28	-	>3	Spontaneous abortions in 14.3%, vs 6.8% in controls; premature births in 8.6%, vs 2.8% in controls	61

TABLE III-1 (CONTINUED)

EFFECTS OF OCCUPATIONAL EXPOSURE TO CARBON DISULFIDE  
PLUS HYDROGEN SULFIDE

No. of Workers	Age: Mean or Range	Concentration (mg/cu m)*		Duration: Mean or Range (years)	Effects	Ref- erence
		CS2	H2S			
209	20-40	>22	<10	-	Menstruation irregular, painful, abundant, and prolonged	60
60	25	ca 16	-	-	Muscular power diminished, reflexes slowed	57
500	18-60	<9	-	1-30	Retinal degeneration, conjunctival inflammation, temporary corneal opaci- ties, color-vision dis- turbances	67
500	20-40	4 ±	<10	-	Menstruation abundant, painful, prolonged	60
94	18	3-9	<10	<1	Hypotension; nervous sys- tem excitability	54

\*1 mg/cu m = 0.321 ppm

\*\*These studies are based on the same cohort of workers, exposed to carbon disulfide plus hydrogen sulfide at concentrations averaging 29-88 mg/cu m in the 1960's, 59-118 mg/cu m in the 1950's, and higher before 1950.

\*\*\*Hydrogen sulfide concentrations are included in those given for carbon disulfide and were estimated to be about 10% of the total.

TABLE III-2

EFFECTS OF EXPOSURE TO CARBON DISULFIDE  
OR TO CARBON DISULFIDE PLUS HYDROGEN SULFIDE ON ANIMALS

Route of Exposure	Species	Exposure Concentration*		Exposure Duration	Effects	Reference
		CS <sub>2</sub>	H <sub>2</sub> S			
Inhalation	Rat	2,330	0	6 hr/d 5 d/wk 10 wk; then 3 d/wk 12 wk	Lethargy, loss of motor control, slowed MCV's with no recovery in 12 wk	78
"	"	2,330	0	6 hr/d 5 d/wk 2-5 wk	Lethargy, slowed but reversible MCV's	78
"	"	2,000	0	2 hr/d throughout pregnancy	Increased fetal mortality, decreased fertility	81
"	"	1,500	0	5 hr/d 6 d/wk 1-15 mon	Weakness, paralysis, myelin and neuron degeneration, weight loss	79
"	"	12	**	70-110 d before mating and during pregnancy	Increased fetal mortality, terata	88
"	"	1.0 1.0 0.1 0.1	0.1 0 0.1 0	160 d	Inflammation of bronchi, weight changes, increased serum asparatate aminotransferase and blood cholinesterase activities; most severe with combined exposures	85

TABLE III-2 (CONTINUED)

EFFECTS OF EXPOSURE TO CARBON DISULFIDE  
OR TO CARBON DISULFIDE PLUS HYDROGEN SULFIDE ON ANIMALS

Route of Exposure	Species	Exposure Concentration*		Exposure Duration	Effects	Reference
		CS <sub>2</sub>	H <sub>2</sub> S			
Inhalation	Mouse	2,000	0	2 hr/d throughout pregnancy	Increased fetal mortality, decreased fertility	81
"	Rabbit	780- 2,330	0	6 hr/d 5 d/wk 38 wk	Paralysis, CNS damage, slight liver damage, weight loss	84
"	"	930 930 0	140 0 140	30 min/d 120 d	Abnormalities of bone marrow, kidneys, spleen; decreased spermatogenesis, loss of appetite, blood changes; most severe with combined exposure	86, 87
ip	Rat	78	0	4 mon (every other d)	Testicular lesions, no spermatogenesis	80
"	"	78	0	2 mon (every other d)	Decreased number of spermatozoa; blood vessels engorged, walls thickened	80
"	"	39	0	"	No effects	80

\*Concentration given in mg/cu m for inhalation exposures, mg/kg for injections; 1 mg/cu m = 0.321 ppm

\*\*Hydrogen sulfide concentration included in that for carbon disulfide