PAPER NO. 26

# PRELIMINARY RESULTS AND COMPARISON OF JAPANESE, FINNISH, AND UNITED STATES CARBON DISULFIDE MICROANEURYSM STUDIES

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## INTRODUCTION

Retinal angiopathy was first associated with carbon disulfide when Goto and Hotta (1967) found increased incidence of retinal small hemorrhages or microaneurysms, nephropathy, anemia, and a variety of subjective symptoms among workers exposed to carbon disulfide in a rayon plant in Japan (Goto and Hotta, 1967; Goto et al., 1971; Goto et al., 1972). Additional studies in Japan and Yugoslavia supported these findings and demonstrated an abnormal response to corticosteroid-augmented glucose tolerance testing among workers exposed to carbon disulfide (Hernberg et al., 1970; Hotta et al., 1972)

Although there was no information on exposure level in these early reports, and they were often flawed by small comparison groups, there seemed no strong reason to doubt a connection between carbon disulfide exposure and a micro-angiopathy mediated by abnormal glucose metabolism until Raitta (1974) reported results of her examinations of 100 workers exposed to carbon disulfide in a Finnish rayon plant previously described by scientists at the Institute of Occupational Health in Helsinki. (See Figure 1.) Aneurysms were much less common in both the exposed and unexposed groups in Finland than in Japan; no effect of carbon disulfide was found on the prevalence of aneurysms; and there was no effect on blood sugar which could be detected by a standard glucose tolerance test not augmented with corticosteroids. Bias in selection of the Japanese cohort was considered the most likely explanation.

In 1976, Sugimoto, Goto and Hotta (1976) reported a "five-year follow-up on retinopathy due to carbon disulfide". They concluded that retinopathy progressed more in the group that remained exposed and that regression in grade of retinopathy occurred significantly more often in workers removed from carbon disulfide exposure than in those remaining in the same work site. While environmental levels again were not given, they recommended reduction in the Japanese TLV (60 $mg/m^3$, or about 19 ppm) for carbon disulfide.

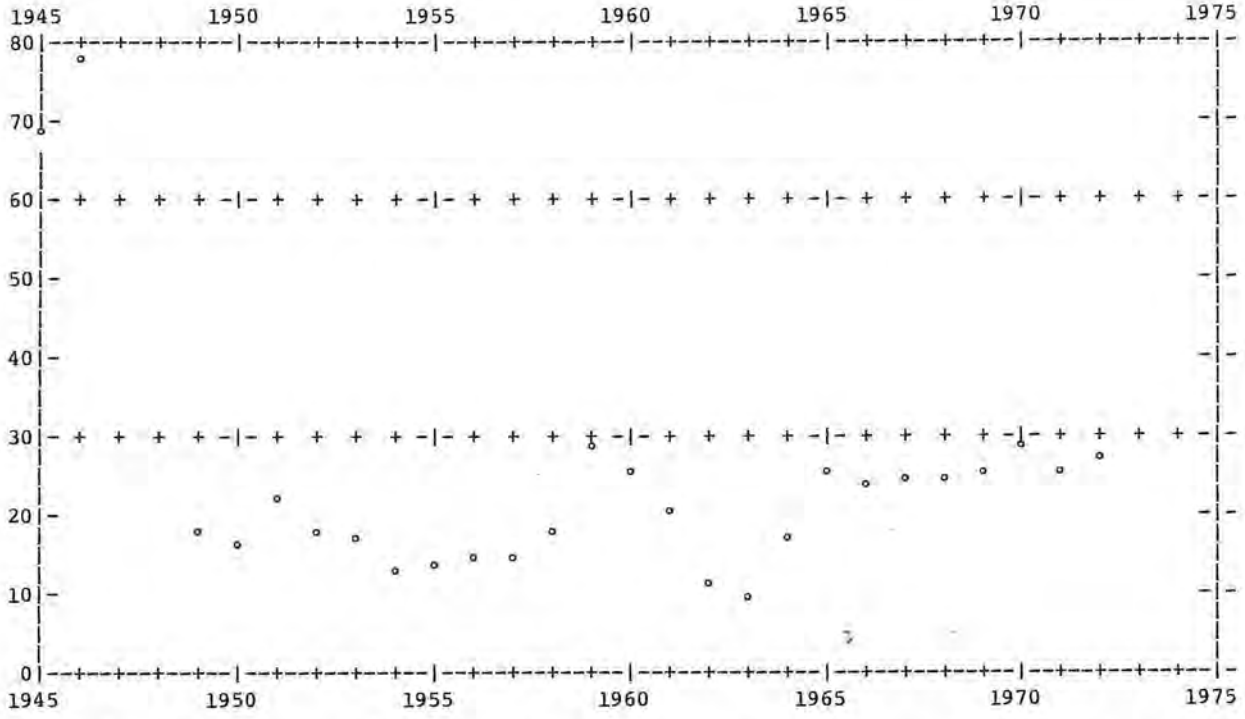


Figure 1. Environmental levels of $CS_2$ in the Finnish rayon plant studied by Cl Raitta, M. Tolonen, & M. Nurminen. 1974. (P. 153) Levels shown are approximately those of the spinning department, which had highe levels than the xanthation room and viscose ripening room, but lowe than the spinning bath room.

By 1977, the Japanese and Finnish groups had combined forces and completed a collaborative study of workers in each country. Environmental levels in the Japanese plant, described in 1976 by Tolonen and others (see Figure 2) were in the same range as those in the Finnish plant (Sugimoto et al., 1977). The ophthalmologic report essentially confirmed the earlier findings that retinopathy was rare in Finland, more common in Japan, and associated with carbon disulfide exposure in Japan but not in Finland (Sugimoto et al., 1976).

About this time, planning was begun for NIOSH's cross-sectional medical and industrial hygiene study of workers exposed to low levels of carbon disulfide. With findings suggesting a problem in Japan and Yugoslavia but no problem in Finland, it seemed prudent to determine where U. S. workers fit in this scheme.

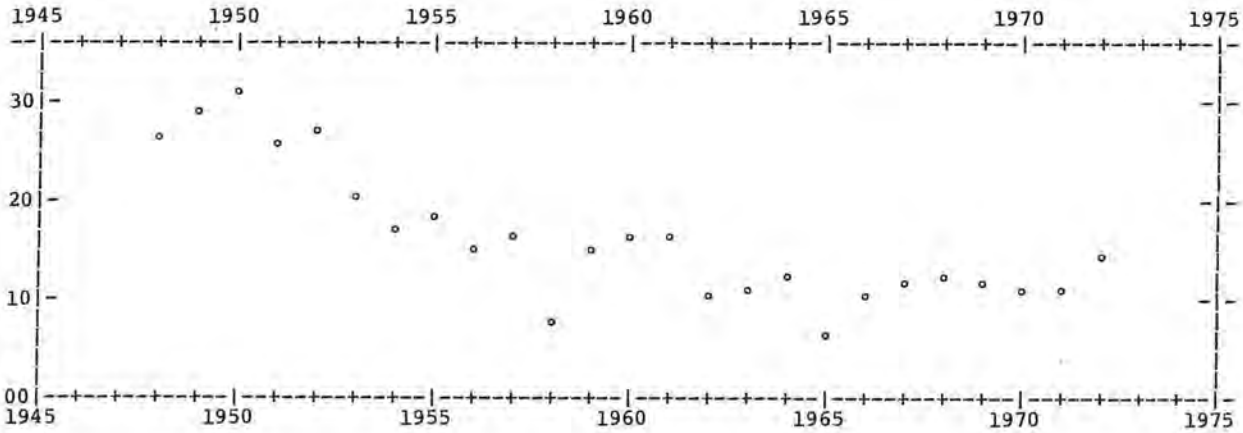


Figure 2. Environmental levels of $CS_2$ in the Japanese rayon plant studied by M. Tolonen, S. Hernberg, C-H. Nordman, S. Goto, K. Sugimoto, & T. Baba. 1976. (P. 253) Values above correspond approximately to the levels for "2nd room" as given in the paper. This room had, in general, the highest levels of the three, but is still not much different from the others.

## METHODS AND MATERIALS

### SELECTION OF A COHORT

After meetings with labor and industry representatives and visits to the four U. S. rayon staple plants, a factory in northeastern Tennessee was chosen as the study site. Rayon staple is a product like cotton wool, used in disposable diapers and sanitary napkins among other products. The plant was opened in 1948 for production of rayon filament (thread or yarn). A viscose rayon staple plant was opened in 1956; a nylon filament plant in 1963; a polyester filament plant in 1966; and a nylon-polyester staple plant in 1967. The rayon filament plant ceased production in 1974 (see Figure 3). The plant was considered among the best controlled in the United States, with environmental area samples going back to 1957 typically showing air concentrations of carbon disulfide between 1.5 ppm and 60 ppm. Levels are generally similar to those noted in Finland and Japan (see Figure 4).

The exposed group was chosen from currently employed members of the viscose rayon staple plant who had been employed in that plant for at least one year. Their employment prior to that time could have included the old rayon filament plant or one of the other synthetic plants listed. Two hundred seventy-three workers were potentially available who fit this description; 189, or 69%, of them signed informed consent forms.

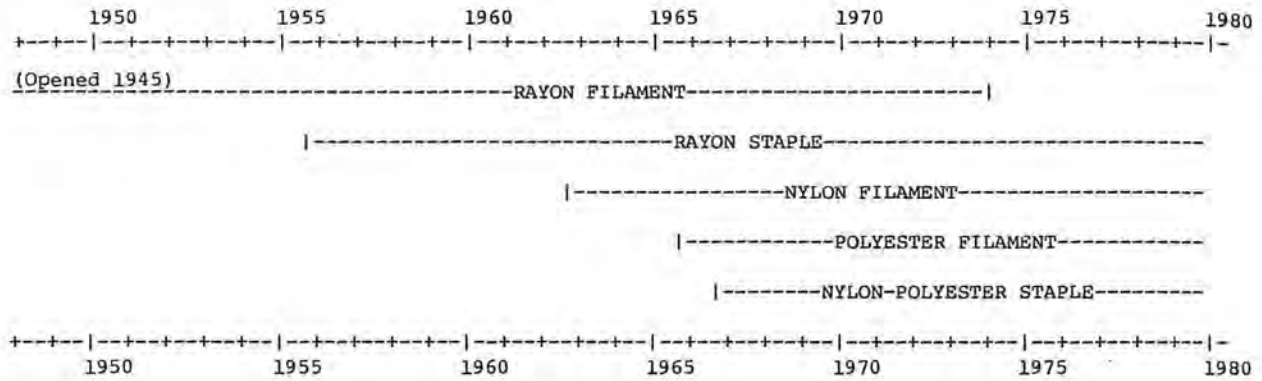


Figure 3. Chronology of plant additions at a site in northeastern Tennessee.

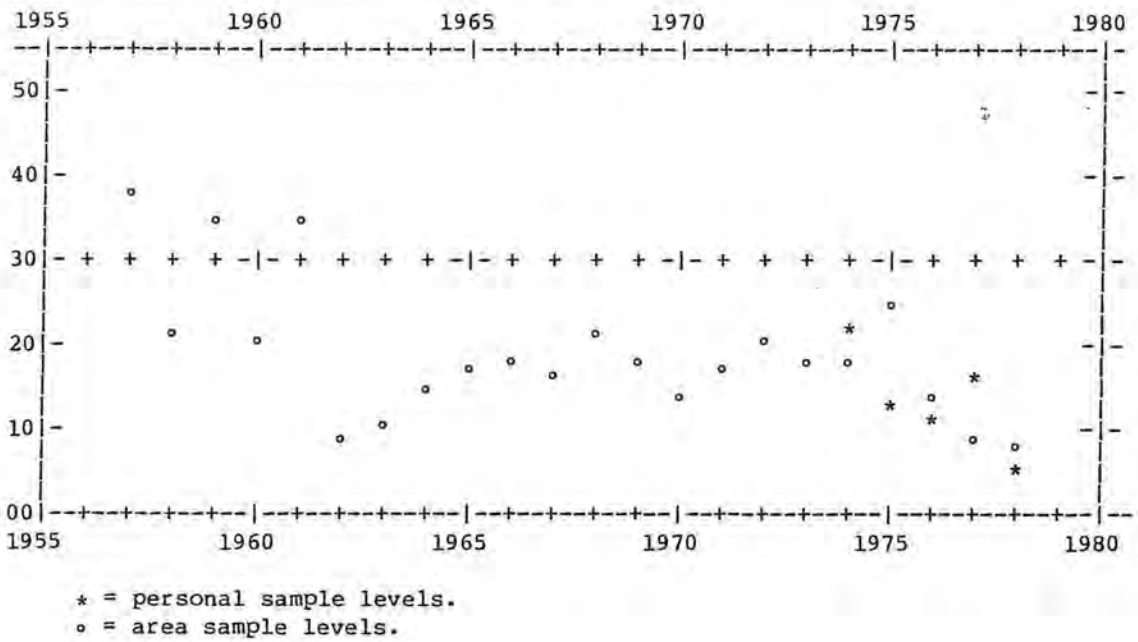


Figure 4. Levels of $CS_2$ in a viscose rayon staple plant in northeastern Tennessee. NIOSH. 1979.

The control group was selected from either the polyester filament, nylon filament, or nylon-polyester staple plant, where there was no occupational exposure to carbon disulfide. Those included had to have been employed at least one year in one or more of these plants, but never employed in either the rayon staple or rayon filament plants. Four hundred twenty-two workers fit this description; 244, or 58%, signed informed consent forms.

Because the incidence and prevalence of cardiovascular disease differ between races, and because there were only twelve non-white employees in the exposed group, only white employees were used for the final analysis. Also lost to final analysis were twenty-one of the exposed workers for whom no exposure data were obtained. One hundred fifty-six exposed and 233 control subjects were used for the final analysis.

## DEMOGRAPHIC COMPARABILITY OF EXPOSED AND COMPARISON GROUPS

General information including name, address, social security number, age, race, and educational level, occupational history (both at the study site and prior to employment at the study site) and smoking history was obtained by questionnaire (which is available on request). See Table 1.

TABLE 1. AGE AND EDUCATION LEVELS OF EXPOSED AND UNEXPOSED WORKERS

| | | Exposed | Unexposed |
|---|---|---|---|
| Age | M | 38.24 | 33.86 |
| | SD | 10.11 | 9.04 |
| Education | M | 10.48 | 11.06 |
| | SD | 2.00 | 1.74 |

## AGE

The average age of the exposed group was 38.2 and that of the comparison group 33.9. This difference was statistically significant using Student's t-test (p less than 0.01).

## EDUCATION

The average number of years of education for the exposed population was 10.5 years and for the comparison 11.1 years. Using Student's t-test this difference was significant at p less than 0.01.

## OCCUPATIONAL HISTORY

Occupational history revealed that there was no significant difference in previous occupational exposed to various potentially hazardous agents between the carbon disulfide exposed and the control groups.

## SMOKING HISTORY

From the questionnaire results, subjects were grouped according to whether they were smokers, ex-smokers, or non-smokers. There was a significant difference between the exposed and control groups when cigarette smoking was considered by pack years. ($p = 0.03$)

## EXAMINATION PROCEDURES

Subjects were seen in a randomized order. No information on exposed history was on forms and examiners were prohibited from asking questions or engaging in conversation which would yield this information. Each subject underwent pupillary dilation with a short acting mydriatic. After dilation was complete, direct ophthalmoscopy was performed and the results recorded. Each subject then had two pictures taken of each retina using a Topcon retinal camera, a monochromatic light source, and panchromatic film. The film was later processed and mounted as black and white slides. Slides were identified only by a randomly-assigned code number and read by ophthalmologists.

## RESULTS

Eye examination results were initially reported as showing or not showing microaneurysms, as showing or not showing hemorrhages, and as showing or not showing other abnormalities. Both exposed and control groups were found to have about 25% prevalence of microaneurysms. Although our technique differed from those in the Finnish and Japanese reports cited earlier, the high prevalence of microaneurysms in the control group was unexpected, even if an association with carbon disulfide exposure exists. Since the concordance between the two independent readers was very good (see Table 2), it seemed possible that the population chosen for the study was, for some reason, unusual. Further inquiry and consultation with additional ophthalmologists revealed that the initial classification system had been too simple; experts advised that a large fraction of the slides could be expected to show small dots that were not clearly aneurysms but were also not clearly artifact. Figure 5 shows a normal retina. Figure 6 shows a retina with definite aneurysms. Figure 7 shows an example of a retina with a dot that is not as easily interpretable and is therefore not likely to be artifact.

TABLE 2. CONCORDANCE OF INITIAL TWO READERS OF RETINAL PHOTOGRAPHS

| | | Reader "M" Microaneurysms | | |
|---|---|---|---|---|
| | | Present | Absent | Total |
| Reader "R" | Present | 107 | 6 | 113 |
| | Absent | 12 | 304 | 316 |
| | Total | 119 | 310 | 429 |

Arrangements were then made to have the slides read by a new ophthalmologist, reporting results separately for each subject's right and left eye as either normal, having definite or uncertain microaneurysms, or having definite or uncertain hemorrhages.

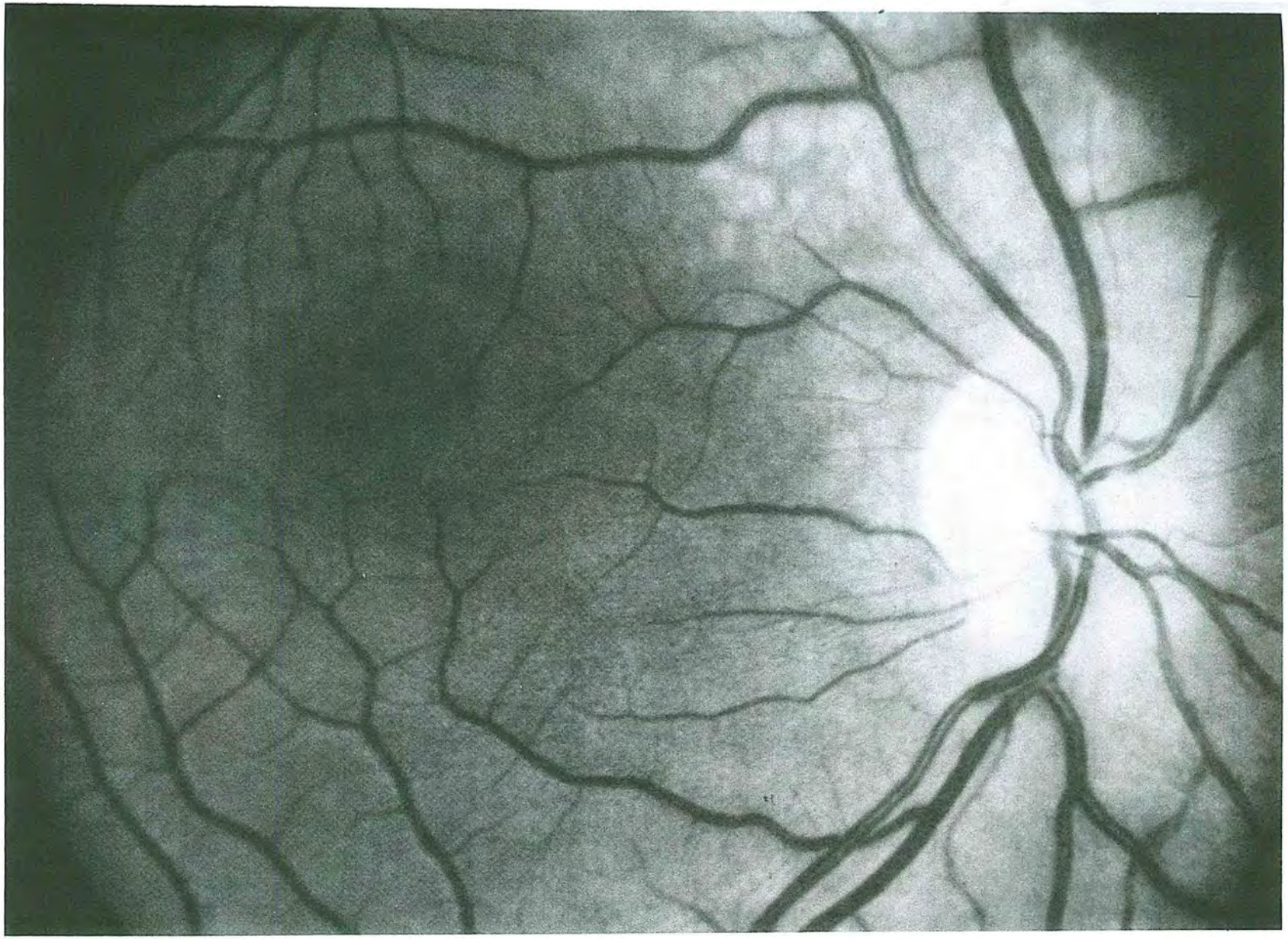

Figure 5. Example of normal retina with no sign of aneurysms.

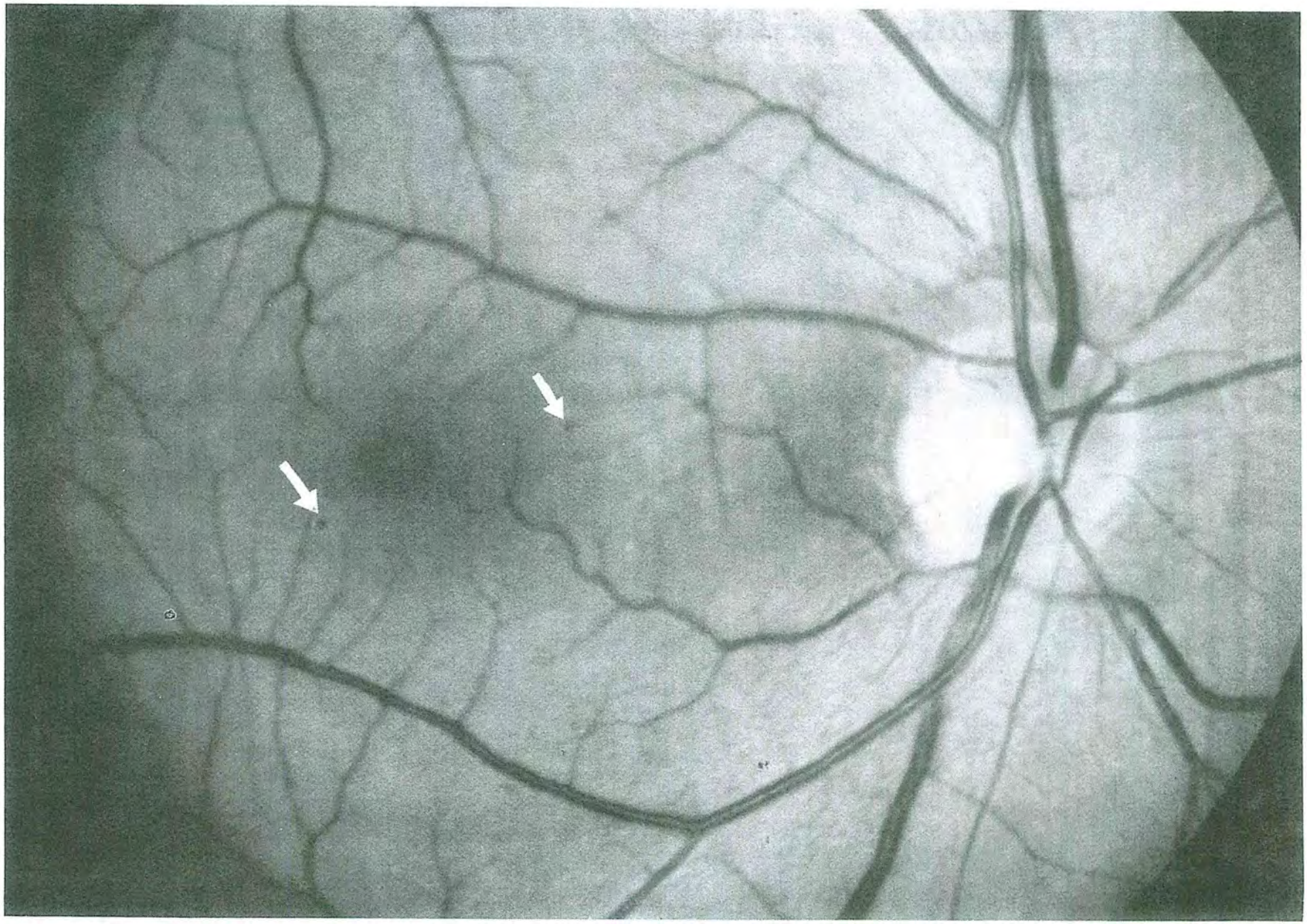

Figure 6. Example of a retina showing definite lesions (arrows).

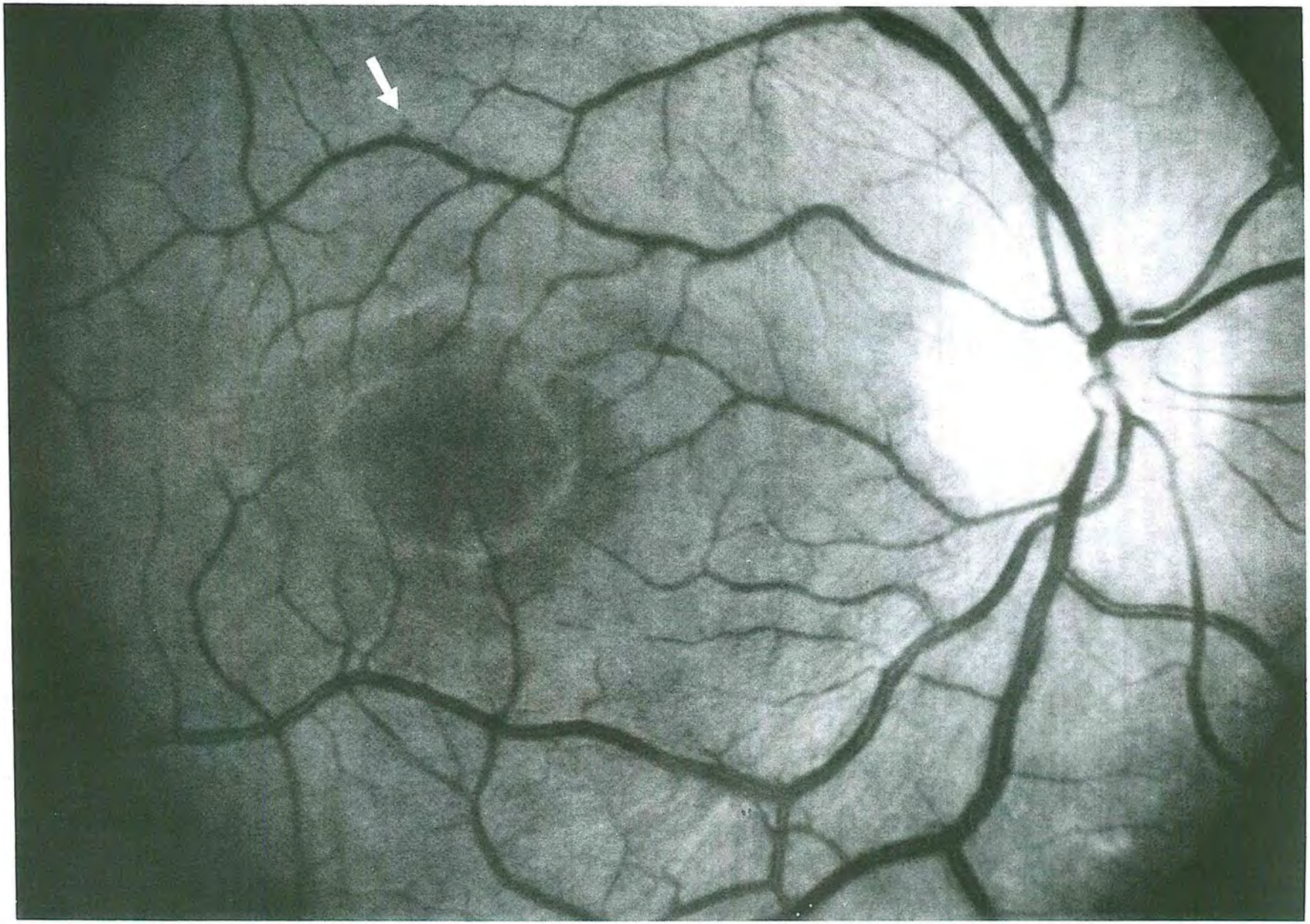

Figure 7. Example of a retina showing signs of a possible aneurysm (arrow).

## RELATIONSHIP OF EYE FINDINGS TO CURRENT EXPOSURE

Table 3 shows results for the control versus exposed analysis, using the second grading method. The exposed group had almost 20 percent retinal microaneurysms (both definite and uncertain) compared to 7.5 percent for the control group (significant at p less than 0.01). The exposed group had 10.5 percent retinal hemorrhages (both definite and uncertain) compared to 3 percent for the control group (significant at p less than 0.01).

TABLE 3. RETINOPATHY (CONTROL VERSUS EXPOSED)

| | Control | | Exposed | | Total | |
|---|---|---|---|---|---|---|
| | N | Percent | N | Percent | N | Percent |
| Microaneurysms | | | | | | |
| Abnormal | 17 | 7.5 | 30 | 19.6 | 47 | 12.3 |
| Normal | 211 | 92.5 | 123 | 80.4 | 334 | 87.7 |
| Total | 228 | | 153 | | 381 | |
| | | | $X^2 = 12.50$ | | p less than 0.01 | |
| Hemorrhages | | | | | | |
| Abnormal | 7 | 3.1 | 16 | 10.5 | 23 | 6.0 |
| Normal | 221 | 96.9 | 137 | 89.5 | 358 | 94.0 |
| Total | 228 | | 153 | | 381 | |
| | | | $X^2 = 8.81$ | | p less than 0.01 | |

Abnormal = definite plus uncertain

For statistical purposes, each job was placed into categories of definitely low exposure (DL, less than 3 ppm), moderate exposure (M, 3 ppm through 10 ppm), definitely high exposure (DH, greater than 10 ppm), and a fourth category, other (O), for a number of workers for whom no exposure data were available. Analysis of prevalence by exposure level, as measured by NIOSH industrial hygienists on two occasions in 1979, shows more retinal microaneurysms and hemorrhages in the exposed group than in the control group at p = 0.04 (see Table 4). There is an apparent increase in incidence of microaneurysms as exposure to carbon disulfide increases from low to medium to high. No such trend is apparent for hemorrhages.

TABLE 4. PREVALENCE OF RETINOPATHY BY EXPOSURE LEVEL

| | Total | | Nonexposed | | Low | | Medium | | High | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | N | % | N | % |
| Microaneurysms | | | | | | | | | | |
| Abnormal | 47 | 12.3 | 17 | 7.5 | 8 | 17.8 | 12 | 18.7 | 10 | 22.7 |
| Normal | 334 | 87.7 | 211 | 92.5 | 37 | 82.2 | 52 | 81.3 | 34 | 77.3 |
| Total | 381 | | 228 | | 45 | | 64 | | 44 | |
| | | | | | | | $X^2$ = 13.08; p less than 0.01 | | | |
| Hemorrhages | | | | | | | | | | |
| Abnormal | 23 | 6.0 | 7 | 3.1 | 5 | 11.1 | 7 | 10.9 | 4 | 9.1 |
| Normal | 358 | 94.0 | 221 | 96.9 | 40 | 88.9 | 57 | 89.1 | 40 | 90.9 |
| Total | 381 | | 228 | | 45 | | 64 | | 44 | |
| | | | | | | | $X^2$ = 9.01; p = 0.03 | | | |

## RELATIONSHIP OF EYE FINDINGS TO CUMULATIVE EXPOSURE

Analysis of the relationship of eye findings to cumulative or long-term exposure is incomplete.

## FASTING BLOOD SUGAR (FBS)

Results of the fasting blood sugar survey are shown in Table 5. As seen, there was no statistically significant difference in FBS between groups in this study.

TABLE 5. FASTING BLOOD SUGAR (FBS)
(CONTROL VERSUS EXPOSURE GROUP)

| | Control | Exposed |
|---|---|---|
| N | 212 | 145 |
| FBS (mg/dl) | 84.27 | 86.08 |
| Stand. Dev. | 11.85 | 11.56 |

P Value = 0.15

## DISCUSSION

Fluorescein angiography is by far the best technique for absolute determination of the type of pathology thought to be associated with chronic carbon disulfide exposure (i.e. microaneurysms and/or small point hemorrhages). This information was known to Sugimoto et al. (1976) when they decided to use only color fundus photography for their study and to record the presence or absence of "small red dots" which comprised both microaneurysms and hemorrhages. They were satisfied that their accuracy of detection of relative retinal abnormalities was as good as fluorescein angiography or the more elaborate systems of grading retinopathy used in Sugimoto's 1976 study.

Fluorescein angiography was not seriously considered for this large scale survey of 446 subjects, despite the evidence to suggest that it produces the best sensitivity and specificity. Time constraints and safety concerns in the field ruled against its use. Retinal photography with monochromatic light source was chosen based on information from Delori and Gragoudas (1977) that claimed "excellent demonstration of the retinal vasculature by monochromatic light at 570 nm". The method used to report findings from the retinal slides depends on the method of photography; where Sugimoto's study (1976) made decisions of severity based on "small red dots", this study made decisions of definite or uncertain microaneurysms and hemorrhages based on "small red dots".

The statistical analysis by control versus exposed shows that the carbon disulfide exposed subjects have a statistically significant increase in microaneurysms when compared to control subjects ($p < 0.01$). The same relationship occurs when hemorrhages are considered ($p < 0.01$). These data seem to confirm the experience of the Japanese researchers who found increased incidences of retinal microaneurysms in $CS_2$-exposed workers but contrasts with the experience of the Finnish researchers, who found no increase. The actual proportions are about half as great as those reported in Japan but about equal to those found in Yugoslavia.

The Japanese researchers and others consider diabetic vascular changes to be the pathogenic mechanism underlying retinal changes in carbon disulfide-exposed workers. The lack of difference in FBS between exposed and control groups does not rule out a pre-diabetic state in our exposed workers, since the fasting blood sugar is a rather poor test for that disorder. Glucose tolerance testing (GTT) gives better clinical information about pre-diabetic subjects; and the prednisolone GTT used by Goto et al. is considered even more sensitive; but such testing was not feasible due to the large size of the population and time and location constraints in this NIOSH study.

These findings, while preliminary and incomplete, suggest that low level exposure to carbon disulfide is an etiologic factor in development of retinopathy among U. S. white male workers. Just as Sugimoto et al. were left with no explanation for the differences between Japanese and Finnish workers, we can offer no new conjecture explaining the difference in response between American and Finnish workers. Completion of analysis by cumulative exposure may suggest an explanation based on differences in exposure, but the difference shown so far does not appear to be clinically important. Analysis of the relationship between serum lipids, also measured during this study, and presence or absence of retinal changes may help define the pathogenesis of the abnormalities.

The wider biological significance of retinal changes is also not as clear as one would wish. Their presence is certainly not reassuring, particularly when seen in the light of many studies showing an excess of cardiovascular deaths (Hernberg et al., 1970) in carbon disulfide workers and of some studies showing significant renal vascular disease as well, but they are not, of themselves, a disease. If these findings withstand the analyses still pending, they will constitute good support for the proposed revision in U. S. permissible exposure levels downward from 20 ppm. Since the group thought to have exposures less than 3 ppm had a prevalence of both aneurysms and hemorrhages

not significantly different from the medium and definitely high exposure groups our results provide no assurance that the NIOSH-proposed 1 ppm standard will be safe. We are reasonably sure a cohort of workers in the United States who have long-term uncomplicated exposures to carbon disulfide in the range of 1-3 ppm cannot be found, so future research should be directed toward determining the pathophysiology of these changes, explaining the differences in responses between nations, and determining upper limits of safe exposure through animal experimentation.

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Subjects of presentations and discussions included behavioral consequences of exposure to occupational environments, development of emphysema after exposure to chemicals, epidemiology of industrial solvents and genetic factors in susceptibility to environmental agents. The toxic effects of specific fuels and chemicals used by the Armed Forces were also discussed.

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