Efficacy of Urinary Monitoring for 4,4′-Methylenebis(2-Chloroaniline)

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The aromatic amine 4,4′-methylenebis(2-chloroaniline) (MBOCA), an animal carcinogen, is used commercially as a curing agent for isocyanate-containing polymers. It is structurally similar to other aromatic amines that cause bladder cancer in occupationally exposed workers. Since the late 1970s, MBOCA users have relied on urinary monitoring as the primary method of assessing MBOCA exposure in the workplace. This paper (1) outlines uncertainties about MBOCA's metabolism in humans that complicate interpretation of urinary MBOCA results; (2) describes alternative laboratory techniques for measuring MBOCA in urine; and (3) discusses observations from site visits concerning the practical application of urinary monitoring. Recommendations to improve the efficacy of monitoring programs for urinary MBOCA are outlined.

The aromatic amine, 4,4′-methylenebis(2-chloroaniline) (MBOCA) is used commercially as a curing agent for cast polyurethane products. Experience with urinary monitoring for MBOCA illustrates the problems and limitations, as well as the valuable aspects, of biomonitoring for assessment of exposure to hazardous agents. MBOCA is of particular interest because the polyurethane industry has adopted a voluntary program of urinary monitoring as the primary method of evaluating MBOCA exposure in the workplace.[1]

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Background

MBOCA, whose structure is shown in Fig. 1, is an animal carcinogen and is structurally similar to other aromatic amines, such as benzidine, which are known human bladder carcinogens.[2] There have been no adequate studies to evaluate the carcinogenicity of MBOCA in humans. The National Institute for Occupational Safety and Health (NIOSH), in a Special Hazard Review published in 1978, recommended that MBOCA be treated as a potential human carcinogen.[2] In 1974 the Occupational Safety and Health Administration (OSHA) issued a standard for MBOCA that regulated the chemical as a potential human carcinogen. However, in the following year, the standard was remanded for procedural reasons and has never been resinstated.[3,4] Because of concern about the potential health effects of MBOCA, many companies have instituted either environmental controls and/or biological monitoring to assess the extent of worker exposure.[1]

MBOCA is used as a curing agent for isocyanate-containing polyurethane polymers. Most uses involve specialty operations in small shops or departments manufacturing industrial products such as polyurethane gears, gaskets, belts, and rollers, and consumer products such as sport boots and roller skate wheels.[5] MBOCA may be purchased in either liquid emulsions or solid pellets; the solid form represents the greater exposure potential because it can lead to dermal contact with settled dust.

No completely satisfactory method exists for monitoring the workplace environment for MBOCA. Air sampling does not accurately reflect worker exposure because it does not estimate the fraction absorbed by the skin, the primary route of exposure. Therefore, high levels of urinary MBOCA may be observed among workers in areas with no demonstrable air concentrations.[6] Wipe sampling can be useful in identifying contaminated work areas for cleanup but only indirectly reflects actual

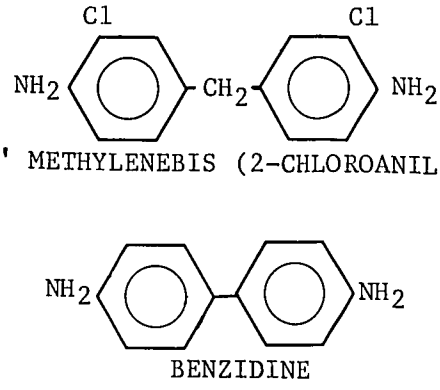

Fig. 1. Structure of 4,4′-methylenebis(2-chloroaniline) and benzidine.

worker exposure. Industry has, therefore, turned to urinary MBOCA measurement as the primary method of monitoring occupational exposure to MBOCA.[1]

Analytic Technique

In MBOCA monitoring programs, an untimed urine sample is collected at the end of a work shift towards the end of the work week. The urine samples are shipped frozen to a laboratory for analysis. At present, very few commercial or government laboratories routinely analyze urine samples for MBOCA. Available analytic techniques are shown in Table 1.

The first method listed in Table 1, thin-layer chromatography (TLC), has been used by the commercial laboratory that services the largest number of polyurethane companies (unpublished data). The TLC method was developed as a quick screening method and is only semiquantitative. In this method, color development in standard plates is compared with color development in sample plates by eye. In contrast, gas chromatography-electron capture (GC-EC), the method recommended by NIOSH in its *Manual of Analytical Methods*,[7] is considerably more time-consuming and costly. The advantage of GC-EC over TLC is that it yields quantitative determinations and its limit of detection is below 1 μg/L. The NIOSH method is not used routinely by the few commercial and state laboratories analyzing MBOCA. The third technique in use is high-performance liquid chromatography (HPLC), a method less time-consuming than GC-EC.

Our interest in urinary MBOCA analysis stems from an effort to characterize MBOCA exposures among US workers on the basis of urinary MBOCA data. To interpret these data, we wanted to compare analytic methods in commercial use with the GC-EC method recommended by NIOSH. We began by contacting the commercial laboratory that had developed the TLC method, because it appeared that this laboratory processed the majority of samples analyzed for urinary MBOCA in the United States.

In a preliminary evaluation of the agreement between the TLC and the GC-EC method, the commercial laboratory split 15 samples which they had received for urinary MBOCA analysis, and shipped an aliquot of each to the NIOSH laboratory for analysis by GC-EC. As shown in Table 2, we found considerable disagreement between the results of the TLC and GC analyses, and nine of the 11 disagreements resulted because the TLC method had lower results than the GC-EC method. We also compared the results of GC-EC analysis in the NIOSH laboratory and a commercial laboratory, using only five samples. Although the results obtained in the two laboratories did not agree, the disagreement did not appear to lie in one direction or the other. These results are very preliminary, and the discrepancies between methods and laboratories possibly arose from problems in splitting, shipping, and storing samples rather than from differences in the analytic techniques. Additional studies are in progress to clarify these issues. The commercial lab that developed the TLC method has simultaneously been conducting independent studies comparing the TLC method with an HPLC technique. Based on our preliminary results and on thir own work, the laboratory has decided that the TLC method is insufficiently sensitive and is in the process of implementing an HPLC method.

Experiments comparing analytic results in different laboratories are useful in quality control programs for highly specialized biological measurements. Some laboratory certification programs require participation in periodic tests of performance in specific analyses. For example, accreditation of laboratories by the American Industrial Hygiene Association requires participation in NIOSH's Proficiency Analytical Testing (PAT) program.[8] This program involves analyzing approximately 25 different samples on a quarterly basis. These samples are comprised of materials commonly analyzed in industrial hygiene laboratories, such as asbestos. The PAT

TABLE 1
Analytic Techniques for Measuring 4,4′-Methylenebis(2 chloroaniline) in Urine

Type of Analysis	Quantity of Urine	Limit of Detection
Thin-layer chromatography (screening technique)	10 mL	10 μg/L
High-performance liquid chromatography		
With UV detector	Variable	30 μg/L
With electrochemical detector	10 mL	10 μg/L
Gas chromatograph-electron capture	5 mL	1 μg/L

TABLE 2
Preliminary Results: Interlaboratory Comparison Study

Laboratory 2 Using Thin-Layer Chromatography	Laboratory 1 Using Gas Chromatograph-Electron Capture		
	<10 μg/L	10-100 μg/L	>100 μg/L
<10 μg/L (nondetectable)	3	7	2
10-100 μg/L	2	1	0
>100 μg/L	0	0	0

program would not test the laboratory's performance on the more unusual analyses they perform. A specific program has been set up by the Centers for Disease Control to test proficiency in blood lead analysis, a relatively more common biological screening technique.[9] In the absence of a centralized quality control program for a specific biological measurement, it would be useful for laboratories to trade samples on a periodic basis as a check on their internal quality control.

Guidelines for Interpreting Sample Results

Another important issue in biological monitoring for MBOCA is the scientific basis, or lack of same, for the various proposed guidelines for taking action based on sample results. Generally, an action level for biological monitoring results is based on (1) an estimate of the total dose or dose to a critical organ represented by the measurement and (2) a dose-effect or dose-response curve for the health effect of most concern. For MBOCA, the estimate of total absorbed dose from urinary concentrations is complicated because the assays measure only parent MBOCA and its glucuronide and sulfate conjugates. For simplicity, this measurable fraction of MBOCA will be referred to as parent MBOCA. The glucuronide and sulfate conjugates are measured as "parent MBOCA" only if the sample is subjected to acid hydrolysis prior to analysis. Most commercial laboratories do not perform acid hydrolysis.

As shown in Table 3, in rats and dogs administered radiolabeled MBOCA by skin application, only 0.4% to 0.5% of the labeled compound appearing in the urine was measurable as parent MBOCA. This finding implies that, at least in those animal species, MBOCA is extensively metabolized, and the amount of MBOCA measured in urine is only a small fraction of the total metabolites present. In fact, urinary parent MBOCA represented only 0.04% to 0.22% of the total absorbed dose.[10,11] It is quite possible that the metabolism of MBOCA in humans is very different from its metabolism in rats and dogs. However, because the animal data are the only data available, it is reasonable to use them to extrapolate from urinary MBOCA concentration to absorbed dose in humans.

A highly simplified model is presented here for estimating the absorbed dose, taking as an example a urinary MBOCA concentration of 100 μg/L, a level chosen because it is the California OSHA standard for urinary MBOCA concentration (Table 4).[12] To calculate the quantity of MBOCA excreted per day, we assumed that an average of 1.3 L of urine are excreted per day, and therefore, 100 μg/L in an untimed urine sample would correspond to a total daily urinary excretion of about 130 μg/day. If this measured amount represented 1% of the total dose absorbed, and in animals this ratio was actually considerably less than 1%, then the total daily absorbed dose of MBOCA would have been at least 13,000 μg or 13 mg.

The information necessary to relate these dose levels to cancer risk in humans is not available; there are no good epidemiologic studies from which a dose-response curve can be derived. The EPA recently performed a risk assessment for MBOCA utilizing dose-response data from a carcinogenesis bioassay in rats (Reference 5; personal communication with J. Springer, Environmental Protection Agency [EPA], 1984). Figure 2 shows the number of excess neoplasms expected per 1,000 workers exposed to MBOCA for a 40-year working lifetime, in relation to the yearly dose of MBOCA absorbed, based on the EPA risk assessment.

Absorption of 14,699 mg of MBOCA per year, the highest dose for which a risk estimate was reported by the EPA, was found to pose a cumulative risk of 68 excess neoplasms per 1,000 workers exposed to MBOCA over a 40-year working lifetime. Exposure to an estimated absorbed dose of 13 mg/day (or 3,250 mg/year), such as was estimated to occur at the present California action level, was expected to cause an excess tumor risk of about 15 neoplasms per 1,000 workers exposed to MBOCA over a 40-year working lifetime.

TABLE 3
4,4′-Methylenebis(2 Chloroaniline) (MBOCA) Metabolism after Skin Absorption in Rats and Dogs

	Rats[10] (72 h)	Dogs[11] (24 h)
Total C-14-labeled compound present in urine as parent MBOCA (%)	0.4	0.5
Total absorbed dose (C-14 labeled compound excreted in feces or urine or retained in carcass) present as parent MBOCA in urine (%)	0.04	0.22

TABLE 4
Calculation of Dose per Year From Microgram 4,4′-Methylenebis(2 Chloroaniline) per Liter of Urine

100 μg/L × 1.3 L/d = 130 μg/d
If this represented 1% of the total dose absorbed:
Total dose per day = 130 μg/d × 100 = 13,000 μg/d = 13 mg/day
Assuming 250 working days per year, absorbed dose per year = 13 mg/d × 250 d/yr = 3,250 mg/yr

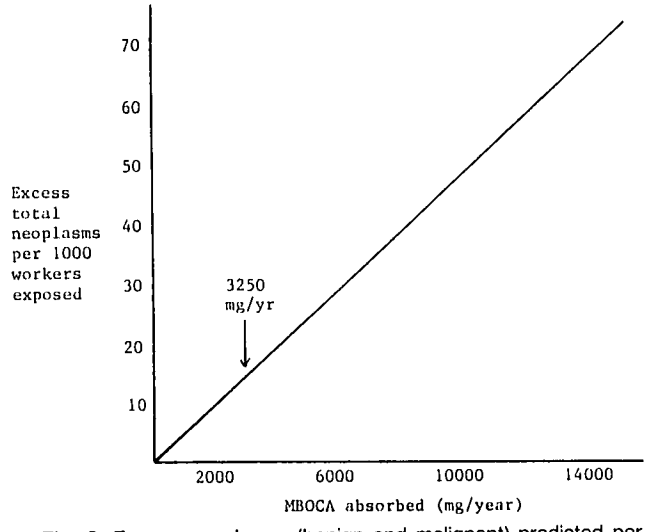

Fig. 2. Excess neoplasms (benign and malignant) predicted per 1,000 workers exposed to 4,4′-methylenebis(2-chloroaniline) for a 40-year working lifetime, by yearly dose absorbed.

Given the tenuous assumptions necessary to estimate absorbed dose and perform risk assessments for humans based on animal data, this graph should not be interpreted as a quantitative prediction of the numbers of tumors that will occur among MBOCA workers. First of all, few workers will be employed in heavily exposed MBOCA jobs for the entirety of a 40-year working lifetime. Second, if MBOCA behaves like other aromatic amine carcinogens in humans, the excess tumors will be almost entirely confined to the bladder, and the best animal model will be dogs rather than rats. The only carcinogenesis bioassay for MBOCA in dogs had a sample size too small to perform a risk assessment; nonetheless, five of six dogs developed bladder tumors.[13]

The point of discussing these risk assessments is *not* to say that the data are sufficient or the models satisfactory for determining a safe concentration of MBOCA in urine. Rather, the present data are insufficient to substantiate the safety of the present day action level for urinary MBOCA of 100 μg/L, which is enforced by California OSHA and used as an informal guideline in other areas.

Planning of Sample Collection

Another important issue in planning a urinary monitoring program for MBOCA and other agents is the frequency with which biological samples should be collected. In determining the frequency, one should consider the kinetics of excretion of the chemical and whether the exposure is continuous or intermittent. These factors influence the degree of fluctuation in the measurement over a period of time.

The optimal frequency of sample collection also depends on the purpose of the program. If the purpose is to monitor individual workers, it may be necessary to obtain urine samples from each worker at frequent intervals. If the purpose is to monitor the workplace, a representative sample of workers at less frequent sampling intervals may suffice.

For MBOCA, the half-life in humans is unknown. In rats administered MBOCA by dermal absorption, the amount of parent MBOCA excreted decreases rapidly within 72 hours, at which time approximately 50% of absorbed dose is still retained in the carcass.[10] Exposure to the chemical may be continuous or intermittent depending on the plant and process. We have not conducted a systematic survey of plants using MBOCA to determine the frequency of biological sample collection. The main commercial laboratory analyzing MBOCA reports that many companies submit samples monthly or quarterly. If the pattern of excretion of MBOCA in humans is similar to that in rats, these infrequent sampling intervals may miss important trends in exposure of workers or the occupational environment. It would therefore be useful to conduct empirical studies at each workplace to determine the variability in MBOCA excretion under normal conditions before determining the sample frequency.

Medical Removal Criteria and Issue of Medical Removal Protection

Another issue that should be considered in setting up a biological monitoring program is the determination of what measures are appropriate when action levels are exceeded. Biological monitoring results indicating excessive exposure may initiate engineering or other environmental controls. They may also result in medical removal of workers who were actually or potentially overexposed.

If medical removal is included as part of a biological monitoring program, consideration should be given to protecting the worker's wages and seniority rights during the period when medical removal is required. For example, under the lead standard promulgated by OSHA in 1978,[14] employers are required to remove the employee from the workplace whenever biological monitoring reveals an abnormally high blood lead level. When this occurs, the employer must guarantee that the removed worker will retain for 18 months the earnings, benefits, and seniority rights of the job from which he was removed.

Ambiguity about how to interpret the results of urinary monitoring for MBOCA complicates the use of such data for regulatory purposes and for medical removal. For example, in interpreting urinary MBOCA levels determined by the TLC method, one should consider that the results are approximate rather than exact. Even for the rigorous, quantitative methods such as GC-EC, extraneous factors may influence the result. For example, urine concentrations of MBOCA reported by the major commercial laboratories were not corrected for dilution of concentration and may vary by one- to fourfold, depending upon urine dilution.[15] The California OSHA Standard requires that specific gravity be adjusted to 1.024.[8] An alternative correction, suggested by NIOSH, is for creatinine concentration.

One author has hypothesized that there may be genetic variation in MBOCA metabolism and excretion in humans.[6] Although not proven, it is quite plausible that such genetic variation exists and could influence urinary concentrations among individuals. The metabolism of aromatic amines involves a large number of enzyme systems, among them the cytochrome P-450 mediated monooxygenases.[16] The inducibility of this enzyme system by exogenous agents shows genetic variation in humans.[17] Humans also show a tenfold variation in urinary β-glucuronidase[18]; this enzyme releases parent MBOCA from its glucuronide conjugates, thereby increasing the yield of parent MBOCA measured in the usual assays, which do not employ acid hydrolysis. Undoubtedly, other enzymes in the pathway also show genetic variation, which could influence the proportion of unmetabolized MBOCA appearing in the urine. Because the assays appear to measure less than 1% of absorbed MBOCA that has not been metabolized to other compounds, any genetic differences resulting in different degrees of metabolism among individuals may affect the result.

Use of Screening Data for Epidemiologic Research

Another important issue in biological monitoring programs is how the data that are collected can and should be used. Biological monitoring data can be a valuable epidemiological tool if the results are systematically recorded and analyzed. Within a specific company, these data may be used to document trends in exposure over a period of time and to monitor the effects of instituting controls. In some instances, the biological monitoring data can be correlated with the results of medical screening exams to yield information on both exposure and outcome.

There may be opportunities to establish industrywide registries by maintaining information from biological measurements at a centralized location. Such a centralized data base would be particularly useful for chemicals like MBOCA, for which most users are small firms where it has been difficult to assemble a cohort for epidemiological study.

An opportunity presently exists to establish such a registry of persons exposed to MBOCA. The major firm supplying MBOCA urine analyses for US polyurethane manufacturing companies has analyzed urine samples from about 50 companies over the past 3 years. A sister company, located in the same facility, provides medical screening services for many of the same companies. If urinary MBOCA concentrations could be linked with the medical screening data, and if, in addition, a limited work history could be obtained, an invaluable registry of workers exposed to MBOCA could be created. The registry would establish a cohort for a prospective epidemiologic study. It would also be useful to public health agencies seeking to document protection afforded by different methods of exposure control. Cooperation of a private research institution or public health agency in setting up the registry would be helpful in providing independent quality control and analysis of the data collected.

Conclusion

Although MBOCA is only one chemical of limited commercial use, some general lessons can be learned from problems that have been encountered in establishing biological monitoring programs for this chemical. Industry originally turned to biological monitoring as the primary method of monitoring MBOCA exposure in the workplace because of difficulties with air and wipe sampling. Institution of biological monitoring programs represents a positive concern on the part of management and is likely to be coupled with other efforts to improve health and safety conditions for employees. The danger of such programs, if they are not scientifically sound, is that they may provide false guidance to both workers and management regarding levels of exposure.

In the course of our involvement with urinary MBOCA screening, we have observed a number of problems. First of all, a number of analytic techniques are in use, but good information is not available as to how their results compare. Second, the data appear to be inadequate to establish action levels for MBOCA in urine or criteria for medical removal. Another potential problem is that many companies are sampling at monthly or quarterly intervals, which may be too infrequent to reflect changes in individual exposure or in workplace conditions. Many aspects of MBOCA's metabolism and the degree of variation among workers are not well understood. One factor influencing measured MBOCA concentration is variation in concentration of the urine among workers or in a given worker over a period of time. This source of variation can be controlled by reporting urinary MBOCA concentrations as microgram per gram of creatinine rather than microgram per liter.

Although biological monitoring of MBOCA has promise as a technique for assessment of exposure, caution must be exercised against overreliance on present monitoring programs. It is unlikely that the gaps in our knowledge about MBOCA's metabolism and carcinogenicity will be overcome in the near future. It is important, therefore, that those persons involved in urinary monitoring programs for MBOCA understand the limitations in interpreting the results.

Acknowledgment

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References

1. Polyurethane Manufacturers Association: Comments on EPA's ANPR for MBOCA, 1983. Docket No. OPTS 06029.
2. *Hazard Review of 4,4′-Methylenebis(2-Chloroaniline).* National Institute for Occupational Safety and Health, Dept of Health, Education, and Welfare (NIOSH) publication No. 78-188, 1978.
3. Proposed standard for 4,4′-Methylenebis(2-Chloroaniline). 40 Fed Reg 4932, Feb 3, 1975.
4. *Synthetic Organic Chemical Manufacturers Association v Brennen.* Third Circuit Court of Appeals, 506 F. 2nd 385, 1974.
5. 4,4′-Methylenebis(2-chloroaniline): Initiation of regulatory investigation. Environmental Protection Agency: [40 (RF Part 764] [Opts-62029] Supporting document (PRL-1).
6. Linch AL, O'Conner GB, Barnes JR, et al: Methylenebis-orthochloroaniline (MOCA): Evaluation of hazards and exposure control. *Am Ind Hyg Assoc* 1971;32:803–810.
7. *NIOSH Manual of Analytical Methods.* National Institute for Occupational Safety and Health, P & CAM 342. Dept of Health and Human Services (NIOSH) publication No. 82-100, 1982, vol 7.
8. *Proficiency Analytical Testing Program (PAT).* National Institute for Occupational Safety and Health, Dept of Health and Human Services (NIOSH) publication No. 83-121, 1980.
9. *Proficiency Testing Summary Analysis: Toxicology Blood Lead.* Atlanta, Centers for Disease Control, Dept of Health, Education and Welfare (PHS), 1979, vol 4.
10. Groth DW, Weigel WW, Tolos WP, et al: 4,4′-Methylenebis (ortho-chloro-aniline) (MBOCA): Absorption and excretion after skin application and gavage. *Environ Res* 1984;34:38–45.
11. Manis MO, Williams DE, McCormack KM, et al: Percutaneous absorption, distribution and excretion of 4,4′-methylenebis (2-chloroaniline) in dogs. *Environ Res* 1984;33:234–245.

12. *Title 8: California Administrative Code:* 2-5215, effective May 25, 1979.

13. Stula EF, Barnes JR, Sherman H, et al: Urinary bladder tumors in dogs from 4,4′-methylenebis(2-chloroaniline) (MBOCA). *J Environ Pathol Toxicol* 1977;1:31–50.

14. Standard for Lead. 43 Fed Reg 53007, Nov 14, 1978.

15. Davidson I, Henry JB (eds): *Todd-Sanford Clinical Diagnosis by Laboratory Methods.* Philadelphia, WB Saunders Co, 1974.

16. Committee on Amines, Board on Toxicology and Environmental Health Hazards, Assembly of Life Sciences, National Research Council: *Aromatic Amines: An Assessment of the Biological and Environmental Effects.* Washington, DC, National Academy Press, 1981.

17. Paigen B, Ward E, Steenland K, et al: Aryl hydrocarbon hydroxylase in cultured lymphocytes of twins. *Am J Hum Genet* 1978;30:561–571.

18. Paigen K, Peterson J, Ward E: A genetic component in the determination of human lysosomal enzyme excretion. *Biochem Genet* 1984;22:517–527.

The Conundrum of AIDS

. . . Why . . . is there no promise of finding either a cure or a vaccine in the near future that will prevent infection with the LAV/HTLV-III virus?

Modern techniques in molecular biology have enabled scientists to discover very precisely the chemistry of the virus, but understanding its biological reaction with the host—the key to effective intervention in the process—presents special difficulties because of the type of antibodies it produces.

If a pathogen causes the host organism to produce neutralizing antibodies that effectively attack the pathogen, then scientists can focus attention on the reaction between the two. However, the antibodies produced in response to infection with LAV/HTLV-III are of the non-neutralizing variety, which means they have no demonstrable effect on the virus but exist quite happily side by side. Consequently scientists are given few clues from that line of inquiry, and must look elsewhere for signs of the virus's biological reaction with its host . . .

Scientists are baffled by the fact that LAV/HTLV-III behaves unpredictably in an infected person. Preliminary studies indicate that over two-thirds of those infected and followed for at least five years have developed no clinical symptoms to date. In up to 20% of those infected, the virus has produced progressive ill-health after an incubation period ranging from six months to five years, or else it has shown intermittent bursts of activity followed by periods of dormancy. It is believed that something triggers the activity of the virus, but as yet the cofactor—or cofactors—necessary to create the right conditions for activity remains a mystery. Researchers are looking at the life-styles of AIDS sufferers for clues, as well as at the possibility that the presence of other diseases such as malaria might have some connection.

—From "AIDS: The Search For Clues" in *WHO Chronicle*, 1985, vol 39, p 207.