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SPERM SHAPE ABNORMALITIES INDUCED BY ENERGY-RELATED HYDROCARBONS
AND INDUSTRIAL CHEMICALS - Progress Report - Jan. 1 to June 30, 1979

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**SPERM SHAPE ABNORMALITIES INDUCED BY ENERGY-RELATED
HYDROCARBONS AND INDUSTRIAL CHEMICALS**

Old Title: Morphological Variants in Damaged Sperm

EPA-IAG-D6-E681-AN

Progress Report

Period covered: July 1, 1978 to December 31, 1978

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OUTPUT TITLE: Using existing and new biological screening and testing systems, identify the presence of carcinogenic, mutagenic, teratogenic and physiologic or metabolically toxic agents associated with coal and oil shale extraction, conversion or utilization.

AIM: To further develop and apply the detection of morphologically abnormal mammalian sperm as a rapid, simple, quantitative assay of the pathologic response of the male gonad to toxic agents associated with the recovery, process stream, and emission of nonnuclear sources of energy, with primary attention to substances from in situ coal gasification and in situ coal-shale extraction.

I. Work Accomplished: July 1, 1978 to December 31, 1978

A. Advances in the automation of a very sensitive mouse sperm assay

During the 6 month period covered by this report, we compiled and analyzed the changes in mouse sperm head dimensions with low doses of x-rays. The following abstract was submitted and accepted for presentation at the 10th Annual Meeting of the Environmental Mutagen Society held in the spring of 1979.

As described in this abstract, we have developed a quantitative method that greatly increases the sensitivity of the mouse sperm assay. These findings are now being adapted to the high speed, scanning-microscopic image analyses system presently available in our division (Young, I.T. and Faskowitz, I.L., Localization of cellular structures, IEEE Transaction on Biomedical Engineering BME-22:1, pp 35-40, 1975, also see work in progress section c).

Abstract: Changes in murine sperm head dimensions induced by low doses of x-irradiation. Chemical mutagens and x-irradiation produce morphologically abnormal sperm that can be easily distinguished under the microscope. We are developing sensitive, objective methods to quantitate such changes using direct measurements of the dimensions of the sperm head. Groups of 3 B6C3F1 mice received acute, testicular doses of 0, 30, 60, 90 or 120 rads of x-irradiation. Thirty-five days later 500 eosin-Y stained epididymal sperm were scored per mouse. A dose dependent increase in percent abnormal sperm was found with a 70 rad dose required to double background. For each mouse, 50 sperm were chosen at random, photographed and printed (MAG X 10^4). For each of the 750 sperm head silhouettes, 11 parameters were measured (4 parallel to the tail axis, 3 perpendicular to the tail axis, 2 diagonals, area, perimeter and a calculated shape factor). For each sperm we then calculated its Mahalanobian distance from the grand mean of the 0 rad group and classified as non-normal those sperm that fell outside the upper 5% Chi-squared value of the 0 rad group. The 11 parameters yielded a dose dependent increase in the proportion of non-normal sperm. There is no significant information loss when certain 4 or 5 of the 11 parameters were selected. Typical doses required to double background are about 10 rads. This quantitative method is considerably more sensitive to radiation induced changes than is visual morphological assessment. The method should be readily adaptable to high speed, automated, scanning-microscopic image analysis.

B. Preparation of technical reports

During this 6 month period we prepared four technical reports of our results of the past 3 years. Four manuscripts with the following titles are attached:

1. Effects of methyl methanesulfonate and dimethylnitrosamine on sperm production in Syrian hamster.
2. Strain variations in radiosensitivity of germinal tissue in male mice.
3. Monitoring human semen in the workplace.
4. Sperm-shape abnormalities in mice and humans.

II. Work in Progress:

A. Genetic differences in the induction of abnormal sperm with polycyclic hydrocarbons: the role of aryl hydrocarbon hydroxylase in mice.

Available evidence suggested that the sperm morphology assay in the mouse was an effective indicator of germinal mutagens (as reviewed by Wyrobek and Bruce, 1978). However, these studies were based solely on the results of one murine genotype and thus the generality of these findings to other species and man remained in doubt. Since genotypes can have major metabolic differences which could alter the response of the seminal epithelium to the mutagen we needed to quantitate the role of genotype in the induction of abnormal sperm.

Although genetic differences among mouse strains play a major role in determining the magnitude of abnormal sperm observed after exposure to x-rays we did not know the importance of such differences for the chemical induction of sperm abnormalities especially by chemical agents such as hydrocarbons that require metabolic activation. Genetic differences in aryl hydrocarbon hydroxylase (AHH) activity largely determine the level of cytotoxic and mutagenic activity of polycyclic hydrocarbons in the liver. In studies with the testes, we have found that there also are genetic differences in the magnitude of abnormal sperm observed after exposure of mice to methylcholanthrene (MC) a polycyclic hydrocarbon. The two AHH inducible strains (C57BL/6J and C3H/HeJ) produced dose dependent increases in abnormal

sperm while the AHH noninducible strain DBA/2J is also nonresponsive to induced sperm abnormalities (see previous progress report, January to July 1978).

However, when we studied the effects of MC in the three hybrids of the strains and the effects of benzo(a)pyrene in all six genotypes we found that our understanding of the genetics of AHH metabolism in the liver was not in general predictive of what we found in the testes. The hybrids did not show the increase in sperm abnormalities in response to MC as we would have predicted by the dominant inheritance of AHH induction. B6D2F₁ produced significantly fewer abnormal sperm in response to MC than predicted by the results with a similar dose in C57BL/6, the inducible parent. In a preliminary experiment on the effects of benzo(a)pyrene on these strains and their hybrids we found that DBA/2 a presumed non-inducible strain produced a dramatic response (from 3 to 28% abnormal sperm) when exposed to 100 mg/kg of BP. This response was very similar to that of C3H/HeJ an AHH-inducible strain (see progress report, January to July 1978). These inconsistencies prompt us to conclude that the effects of hydrocarbons on sperm abnormalities cannot be predicted from our present understanding of AHH induction in the liver. We feel the testes must be considered as an organ with its own role in metabolism.

These findings also cast a significant doubt on the gene's linkage between the induction of sperm abnormalities and AHH responsiveness in these strains. We have therefore prepared some 20 males of the backcross genotype C57BL/6 x (DBA/2 x C57BL/6). Such males are either homozygous AHH responsive at the AHH locus or AHH heterozygous, with a responsive and a non-responsive allele. We will test for a relationship between the induction of abnormal sperm with hydrocarbons and the gene dosage of AHH inducible alleles. We will a) hemicastrate each male to determine his baseline level of sperm

abnormalities, b) expose each male to subacute regime of methylcholanthrene (30 mg/kg/day x 5 days), c) determine the AMH responsiveness of each male by progeny typing, and d) determine the effects of methylcholanthrene on sperm abnormalities 35 days after exposure.

B. The effects of a direct acting alkylating agent on rabbit semen.

Rabbits are well suited for studies of the effects of chemical agents on sperm. The rabbit can be easily trained to ejaculate into an artificial vagina so that repeated samplings of the same individual are possible. (In spite of much effort a comparable technique for mice has yet to be found.) Thus rabbits can serve as their own controls when one compares semen samples collected before exposure with those obtained during and after chronic exposures to chemical agents. In addition, rabbit semen is relatively "human-like". Accessory gland secretion can be cleanly removed allowing easy concentration of sperm, and the morphology of rabbit sperm is similar to that of man. Quantitative morphological criteria of defective rabbit sperm are therefore more likely to allow easy extrapolation to human sperm. However, in earlier studies of the effects of methylcholanthrene, ethylenedibromide, epichlorohydrin and Mitomycin-C, we found no chemically related effects on semen even at doses that were clearly toxic (as judged by weight loss, etc).

Comparison of these results with results obtained in the mouse, however, is difficult because the rabbits were exposed chronically and the mice subacutely. We therefore propose to expose rabbits subacutely (1 injection per day for 5 days) to methylmethanesulfonate (MMS), a direct-acting, alkylating agent. We will use rabbits trained for semen collection into artificial vaginas. Groups of two rabbits each will be exposed to 0, a low and a high total dose of MMS with the high dose equal to about $\frac{1}{2}$ the lethal

dose. Rabbit semen will be sampled two weeks before and every two weeks for about 10 weeks after exposure. Semen will be scored for changes in sperm counts, motility and morphology.

RELATIONSHIP TO OTHER PROGRAMS:

Collaboration with other programs within and outside the Division continues to be very strong and productive. The following is a list of collaborators, their programs and the topics of collaboration:

A. Within the Biomedical Division

1. With the funding of B.L. Gledhill's program, "Sperm as Indicators of Hazard" DOE/ASEV RPIS 000254, we have studied the inheritance of sperm abnormalities in F_0 , F_1 , F_2 and backcross generations for 4 strains of mice and we have studied the genetic inheritance of sperm abnormalities in mice carrying reciprocal translocations.
2. In conjunction with B.L. Gledhill and the above program as well as the DOE programs "Flow Systems Application" DOE/ASEV RPIS 000257, and "Flow Systems Development" DOE/ASEV RPIS 000256, we have made use of available state of the art flow technology to automate the quantitation of abnormal sperm.
3. In collaboration with R. Balhorn, "DNA, Chromatin and Chromosomes" DOE/ASEV RPIS 000266 and with the funding of the program "Sperm as Indicators of Hazard" we have found that the sperm nucleoproteins is made up of at least two moieties (see publication No. 6). We are now looking at the abnormal sperm of mutagenized mice to determine whether they differ from the normal in nucleoprotein composition.
4. Fred Hatch, "Toxicology of Coal Gasification" DOE/ASEV RPIS 002631, is coordinating the testing of effluents from in situ coal gasification technologies.

5. Jim Felton and L. Dobson, "Radiosensitivity of Oocytes" DOE/ASEV RPIS 000252 and "The Mouse System as Monitor of Chemical Toxicity" EPA-IAG-D6-E681-AQ, have supplied us with males exposed in utero to x-rays and methylcholanthrene. Together with J. Felton we have also carried out together much of the work reported here on the genetics of AMH induction in relation to the production of abnormal sperm.
6. B. Mayall, "Health Effects - Fossil Fuels" DOE/ASEV RPIS 002629, is coordinating a broad based study of health effects in coal and petrochemical workers. We are planning to evaluate their semen.

B. Outside the Division

1. We have accepted an invitation to participate in Phase II of the ICI/MRC/NIEHS collaborative study on carcinogen/noncarcinogen paired compounds coordinated by Dr. J. Ashby, Imperial Chemical Industries, Ltd., U.K. and Dr. F. de Serres of NIEHS.
2. We have been asked by numerous laboratories, mostly within the USA, to help them score animals for morphologically abnormal sperm or to help them set up the mouse sperm assay. For example:
 - a) Drs. Mahlum and Sikov, Battelle N.W. Laboratory, Richland, Washington to score rat sperm from animals exposed to electromagnetic radiation and plutonium.
 - b) Dr. Arthur Penn, New York University Medical Center, New York to score sperm of roosters exposed to methylcholanthrene.
 - c) Dr. Rainer Lange of Schering Company, Berlin, German to score standard sperm to help him set up the sperm assay at his company.
 - d) Dr. J. Topham of Imperial Chemical Industries Ltd., U.K. to help him set up the sperm assay at his company.
 - e) Dr. D. Brusick and M. Bakshi of Litton Biometrics, Ltd., Maryland to set up the mouse sperm assay at Litton.

- f) Dr. E. Wei of School of Public Health in Berkeley, to help him set up the mouse sperm assay.
 - g) Dr. D. Perrin of the Pasteur Institute in Paris to help that institute initiate a commercially available mouse sperm assay.
3. With W.R. Bruce and A. Hugenholtz of the Ontario Cancer Institute, Canada, we have collaborated on studies of the heritability of sperm abnormalities have have recently published an extensive review of the field of abnormal sperm in mice and humans (see publication No. 3).
4. We have numerous collaborations to analyze the semen of humans occupationally or therapeutically exposed to potentially hazardous agents.
- a) Dr. D. Whorton, Director of Labor Occupational Health Program, U.C. Berkeley, is supplying us with sperm of workers exposed to pesticides.
 - b) Drs. Ellis Cohen and Jay Brodsky, Dept. of Anesthesia, Stanford Medical School, are supplying us with sperm from anesthesiologists.
 - c) Dr. M.L. Meistrich, Dr. E. Steinberger in conjunction with the heads of 4 clinical departments of the M.D. Anderson Hospital and Tumor Institute will supply us with samples of sperm from patients on chemotherapy.
 - d) Dr. Ed Schneider of the National Institute of Aging, Maryland, has sent us the semen of young and old human males so that we can study the effects of age on semen quality.

Publications on project to date:

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5. Wyrobek, A.J., Watchmaker, G., Foote, J. and Singh, H., "The Effects of Methyl Methanesulfonate and Dimethylnitrosamine on Sperm Production in the Syrian Hamsters," in Developmental Toxicology of Energy-Related Pollutants, Malhum, D. and Sikov, M., Eds. (DOE, Oak Ridge, 1978), pp 68.
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EPA-1AG-D5-E681-AN, Comprehensive summary three-year progress report
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9. Wyrobek, A.J., and Gledhill, B.L., "Monitoring human semen in the
workplace," EPA Technical Report (submitted).
10. Wyrobek, A.J., and Bruce, W.R., "Sperm-shape abnormalities in mice
and humans," EPA Technical Report (submitted).

DISTRIBUTION

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