No such cases were encountered in tests covering a wide range of home-usage conditions reported here, or in similar tests among 5,943 U.S. housewives, (Griffith *et al.*, 1969) and 1,995 European housewives (unpublished, Procter and Gamble Ltd.). Further tests are under way and others are planned but the failure to reproduce adverse reactions in the present series of tests, and the lack of confirmatory reports of such cases in the world literature, even though these products have continued to be used by millions of housewives, makes it reasonable to conclude that the addition of enzyme at the low level used in these detergents does not add to their primary irritant potential in use. All the evidence is against enzyme detergents having acted as skin sensitizers in the cases reported (*British Medical Journal*, 1970). Griffith *et al.* (1969) failed to detect any sensitization potential on enzyme products. This suggests that factors other than the enzyme washing

product may have been responsible for the outbreaks reported by Jensen and by Ducksbury and Dave.

References

- British Medical Journal*, 1970, 1, 518.
 Ducksbury, C. F. J., and Dave, V. K. (1970). *British Medical Journal*, 1, 537.
 Griffiths, J. F., *et al.* (1969). *Food and Cosmetics Toxicology*, 7, 581.
 Jensen, N. E. (1970a). *British Medical Journal*, 1, 299.
 Jensen, N. E. (1970b). Personal communication, August 1970.
 Johnson, S. A. M., Kile, R. L., Kooyman, D. J., Whitehouse, H. S., and Brod, J. S. (1953). *Archives of Dermatology and Syphilology*, 68, 643.
 Keay, L., and Moser, P. W. (1969). *Biochemical and Biophysical Research Communications*, 31, 600.
 Liss, R. L., and Langguth, R. P. (1969). *Journal of the American Oil Chemists Society*, 46, 508.
 Smith, E. L., De Lange, R. J., Evans, W. H., Landon, M., and Markland, F. S. (1968). *Journal of Biological Chemistry*, 243, 2184.

Chromosome Breakage and Ultrasound

ELIZABETH BOYD, USAMA ABDULLA, IAN DONALD, J. E. E. FLEMING, A. J. HALL, M. A. FERGUSON-SMITH

British Medical Journal, 1971, 2, 501-502

Summary

Human lymphocyte cultures were examined for chromosome damage after exposure to ultrasound. Control and treated slides were scored "blind" and showed no evidence of damage due to ultrasound. Neither was there evidence of chromosome damage in blood cultures from six infants whose mothers had ultrasound during pregnancy when compared with that from six infants whose mothers had not. Our results suggest that if diagnostic ultrasound causes chromosome damage it does so with less frequency than acceptable levels of diagnostic x-irradiation.

Introduction

Sonar is now an established and invaluable diagnostic tool which has increasing clinical applications particularly in obstetrics and gynaecology. It is therefore of importance to establish that the technique is free from harmful effects to the fetus. Genetic damage is one possible danger, and experience with x-irradiation suggests that this danger may to some extent be gauged by examining insonated cells for chromosome breakage. Macintosh and Davey (1970) reported

studies on human blood cultures exposed for one and two hours to ultrasound from an ultrasonic fetal heart detector. They found an increase in frequency of chromosome and chromatid aberrations and suggested that clinical ultrasound was potentially mutagenic to the fetus. Their findings prompt us to report an entirely different experience with similar experiments carried out in this hospital in 1966 and referred to briefly elsewhere (Donald, 1969).

Blood cultures consisting of 0.5 ml of whole blood from a healthy adult added to 4.5 ml of Waymouth's tissue culture medium and 0.1 ml of phytohaemagglutinin were incubated at 37°C in sterile polystyrene containers. Two sources of ultrasound were tested separately in each of three experiments. Source 1 (Doptone I, Smith Kline and French) gave a continuous ultrasound beam at a frequency of 2.0 MHz with an estimated power output at the crystal face of 25-30 mW. Source 2 (Diasonograph, Nuclear Enterprises) gave a pulsed beam of ultrasound at a pulse repetition frequency of 750/sec and was used at maximum output (transmitter attenuation 0 decibels); the transducer frequency was 1.5 MHz. In each case the cultures were placed on top of the ultrasound probe.

Experiments

(1) Cultures were exposed to ultrasound for 13 hours at 20°C and then incubated at 37°C for 72 hours. The container was coupled to the transducer with a layer of oil. Most cells were thus exposed during interphase to at least two cell cycles before harvesting.

(2) Cultures were incubated for 60 hours before exposure to ultrasound for 10 hours at 37°C in a specially constructed water bath, and harvested after exposure to demecolcine without ultrasound for two and a half hours. It was estimated that under these conditions the cell cultures were exposed to at least 60% of the ultrasound energy. The mitoses analysed were therefore derived from cells exposed to ultrasound during most of the cell cycle (including the DNA synthetic phase) before harvesting.

(3) Cultures were incubated for 72 hours at 37°C, demecolcine was then added, and the cultures were exposed to ultra-

University of Glasgow, Glasgow W.2

ELIZABETH BOYD, PH.D., Cytogeneticist, Medical Genetics Unit
 USAMA ABDULLA, M.B., M.R.C.O.G., Research Fellow, Department of Midwifery
 IAN DONALD, M.D., F.R.C.O.G., Regius Professor of Midwifery
 J. E. E. FLEMING, Research Technologist
 A. J. HALL, B.Sc., Research Technologist
 M. A. FERGUSON-SMITH, M.B., M.R.C.PATH., Senior Lecturer in Medical Genetics

sound for four hours at 37°C before harvesting as in experiment 2. The cells analysed were those exposed in mitosis and in the G₂ phase.

Results

Control cultures without ultrasound treatment were set up with each experiment. At the end of an experiment the cells were harvested, fixed, and stained by the routine air-dried technique. The slides were coded so that insonated and control cultures remained unidentified until all slides were scored. Fifty mitoses from each culture were counted and fully analysed by direct microscopy. A further 350 mitoses were examined for chromatid and chromosome aberrations. The results (Table 1) are expressed in terms of the number of cells in which one or more aberrations were detected. A similar low frequency of aberrations was found in each experiment and control, suggesting that the long periods of exposure to ultrasound had no appreciable effect on chromosome breakage under the conditions of the experiment.

TABLE 1—Number of Cells with Aberrations

		Chromatid Aberrations	Chromosome Aberrations*	Total Cells Examined
Experiment 1	Source 1 ..	10	4 (F,F,F,F)	400
	Source 2 ..	14	4 (B,B,F,F)	400
	Control ..	10	4 (B,B,D,F)	400
Experiment 2	Source 1 ..	12	5 (B,B,B,D,F)	400
	Source 2 ..	15	3 (B,F,F)	400
	Control ..	27	2 (B,R)	410
Experiment 3	Source 1 ..	19	1 (B)	400
	Source 2 ..	16	3 (B,B,D)	400
	Control ..	15	3 (B,B,F)	400

* B = One or more chromosome breaks. D = Dicentric chromosome. F = One or more acentric fragments. R = Ring chromosome.

A preliminary attempt was also made to compare the frequencies of "spontaneous" chromosome aberrations in routine blood cultures obtained from infants whose mothers had received diagnostic ultrasound during pregnancy. Chromosome studies on six infants (Table II) were compared with those on six infants matched for age and sex whose mothers had not received ultrasound. No chromosome aberrations were detected (Table III). Experience with chromosome breakage due to diagnostic x rays (Buckton *et al.*, 1962; Conen *et al.*, 1963) suggests that if diagnostic ultrasound does cause

TABLE II—Infants Exposed In Utero to Ultrasound

Infant	Gestation (weeks)	Type of Ultrasound
1	22	Source 2
	26	Source 2
2	40	Source 2
3	35	Source 2
4	24	Source 2
	24	Source 1
5	30	Source 2
6	19	Source 2

chromosome breakage it does so with less frequency than acceptable levels of diagnostic x-irradiation.

TABLE III—Chromosome Analysis of Lymphocyte Cultures from Infants Exposed to Ultrasound In Utero and from Controls

	Distribution of Chromosome Counts				No. of Cells with Chromosome Aberrations
	m - 1	Modal	m + 1	Total	
Infant 1	3	27	—	30	0
" 2	—	25	—	25	0
" 3	—	25	—	25	0
" 4	5	10	—	15	0
" 5	2	18	—	20	0
" 6	—	20	—	20	0
Control 1	1	24	—	25	0
" 2	3	22	—	25	0
" 3	3	47	—	50	0
" 4	3	22	—	25	0
" 5	1	29	—	30	0
" 6	1	23	—	24	0

Discussion

Although the in-vitro experiments recorded here differ in a number of respects from those reported by Macintosh and Davey (1970), it is not at present clear why two similar and independent studies should have quite different conclusions. One suggestion by Coakley *et al.*, (1971) is that the chromosome damage reported by Macintosh and Davey may be due to toxic substances released from polyethylene by ultrasound, rather than the result of ultrasound itself. Apparently opposite results have also been reported by Serr *et al.* (1970), who found an increased frequency of chromosome breakage in fetal fibroblasts subjected to ultrasound, and by Fischer *et al.* (1967), who found no increase in insonated fetal cord blood. One could speculate on other differences in the experimental conditions of these studies to explain these discrepancies. However, further work seems indicated, and it would seem important to investigate the threshold at which ultrasound can produce chromosome damage in vitro, and to obtain more extensive data on the in-vivo effects of clinical ultrasound on lymphocyte chromosomes. Both approaches are currently under study at the Queen Mother's Hospital.

We are grateful to Miss Irene E. Greig for her expert technical help.

References

- Buckton, K. E., Jacobs, P. A., Court Brown, W. M., and Doll, R. (1962). *Lancet*, 2, 676.
- Coakley, W. T., Hughes, D. E., Slade, J. S., and Laurence, K. M. (1971). *British Medical Journal*, 1, 109.
- Conen, P. E., Bell, A. G., and Aspin, N. (1963). *Pediatrics*, 31, 72.
- Donald, I. (1969). *American Journal of Obstetrics and Gynecology*, 103, 609.
- Fischer, P., Golob, E., Kratochwil, A., and Kunze-Mühl, E. (1967). *Wiener klinische Wochenschrift*, 23, 436.
- Macintosh, I. J. C., and Davey, D. A. (1970). *British Medical Journal*, 4, 92.
- Serr, D. M., *et al.* (1970). European Congress of Perinatal Medicine, London, April, 1970.