can be proved by single T_3 and and or T_4 measurements before treatment is started.

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ULTRASOUND AND MAMMALIAN D.N.A.

SIR,—Because of increasing medical use of ultrasound, especially in obstetric diagnosis, it is important to check that it is not genetically harmful.

Most studies of the effect of sonication at medical doses on chromosomes have shown no damage. Nevertheless, the absence of visible chromosomal damage does not exclude the possibility of damage to the D.N.A. molecules. We therefore checked solutions of purified calf-thymus D.N.A. for possible effects of ultrasound treatment applied within a range of frequencies and intensities used in medicine, both in treatment and in obstetric diagnosis. The D.N.A. molecules were photographed in an electron microscope (magnification × 29 000), and the lengths of sonicated and control molecules of D.N.A. were compared.

We found very considerable damage with intensities commonly used for therapy (i.e., 1·5W/cm² and 1W/cm², and also for 200mW/cm²) for various periods. All the D.N.A. molecules were broken down. We are reporting this in more detail elsewhere. We thus found that at the therapeutic doses and at 10 times less, sonication is mutagenic for purified D.N.A.

For doses commonly used in obstetrics (i.e. 20mW/cm², 1MHz), we found no effect. But we have to point out that a dose of 200mW/cm², which has a drastic effect, is only 10 times the intensity used for obstetric diagnosis, which is sometimes applied for a much longer time (for several hours in fetal-heart monitoring during labour). Besides, sonication can have short peaks of higher intensities than those given by calibration of the machine. Although our results cannot lead to a conclusion as far as their in-vivo implications are concerned, we must point out that intensities which are only one order of magnitude higher than those used in obstetric diagnosis, and lower than those used in therapy, cause large-scale fragmentation of purified D.N.A. in solution. Therefore, we believe that, at present, although considerable development of the use of ultrasound in diagnosis is completely justified, exposure should be minimised, especially in obstetric use, because the growing fetus is very susceptible to mutagenic agents.

As for ultrasound doses used for therapy, although they appeared to be mutagenic on purified D.N.A.., the consequences are, a priori, less important than if fetal cells are involved, although it is known that mutations can contribute to cancer ætiology. We think it is important to continue to study the effect of ultrasound at medical doses on D.N.A. in vivo, and we completely agree with Fischman, who emphasises[1] "that it is most important, in working with ultrasound, that all of its variables be specified and, in so far as possible, controlled".

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1. Fischman, H. *Lancet*, 1973, ii, 920.

HORMONE SENSITIVITY AND BREAST CANCER

SIR,—We have been interested for some years in the use of cytochemical techniques to study clinical problems, so we read with interest the letter from Miss Wilson and Dr Carr (Sept. 6, p. 465) on in-vitro testing for hormone sensitivity in breast cancer. The points they raise merit further comment.

We have developed cytochemical methods for the in-vitro examination of breast cancers[1–3] which were modified and applied by Prof. J. R. Hobbs's group.[4–7] More recently we have extended the use of these methods to hormone bioassay,[8] and our results have shown that cytochemistry can be made very precisely quantitative. However, this experience has made clear to us what meticulous attention must be paid to niceties of technique if meaningful results are to be obtained.

Wilson and Carr suggest that variation in section thickness may give rise to errors in the interpretation of results. In fact, consideration of the principles underlying tissue densitometry indicates that such variation will completely invalidate the results. These principles are essentially similar to those underlying conventional spectrophotometry: in the one case, light is absorbed by a solution contained in a cuvette; in the other, by a stain precipitated in a tissue section. Thus the thickness of the section is precisely analogous to the path length of the cuvette, which, as the most junior chemical-pathology technician well knows, is directly proportional to the measured optical density. Constant section thickness is therefore absolutely essential.

Unfortunately it is one thing to recognise the necessity of constant section thickness, but quite another to achieve it. Butcher[9] has shown that variation in cutting speed is one cause of inconstant thickness, and we would recommend that normally a cryostat with an automatic cutting device should be used for quantitative work, as this instrument cuts with a uniform speed.

Inconstant section thickness, however, cannot account for the anomaly reported by Wilson and Carr, whereby sections which appeared to the eye to differ in staining intensity showed no difference by densitometry. For, even though the concentration of stain may be equal, if one section is significantly thicker than another it will appear darker to the eye as well as to the densitometer. Similarly, if the difference is apparent optically, it must also be apparent densitometrically. If not, then something is amiss with the densitometric technique.

There are two likely sources of this type of error. One is that the optical densities being measured are simply too high for the instrument to discriminate between them. Just as in spectrophotometry, in tissue densitometry there is a range of optical density above which precision declines, ultimately to be lost altogether as the percentage of light transmitted to the photomultiplier becomes negligible. Optical density must therefore be kept within a suitable range by limiting the incubation time for the enzyme reaction which leads to precipitation of the formazan.

A second source of error is the optical inhomogeneity of the formazan deposit in the tissue. Unless the diameter of the scanning beam of light which is transmitted through the tissue by the densitometer is so small that it approaches the limits of resolution of the optical microscope (i.e., about 0·2 μm) great inaccuracies will result.[10,11] The use of inappropriate instru-

1. Altman, F. P., Chayen, J. *in* The Treatment of Carcinoma of the Breast (edited by A. S. Jarrett); p. 68. Amsterdam, 1968.
2. Altman, F. P., Bitensky, L., Chayen, J., Daly, J. R. *Proc. Ass. clin. Biochem.* 1968, 5, 115.
3. Chayen, J., Altman, F. P., Bitensky, L., Daly, J. R. *Lancet*, 1970, i, 868.
4. Salih, H., Flax, H., Hobbs, J. R. *ibid.* 1972, ii, 1198.
5. Salih, H., Flax, H., Brander, W., Hobbs, J. R. *ibid.* p. 1103.
6. Flax, H., Salih, H., Newton, K. A., Hobbs, J. R. *ibid.* 1973, i, 1204.
7. DeSouza, I., Morgan, L., Lewis, V. J., Raggatt, P. R., Salih, H., Hobbs, J. R. *ibid.* 1974, ii, 182.
8. Symposium on Cytochemical Bioassay, *Clin Endocr.* 1974, 3, 303.
9. Butcher, R. G. *Histochemie*, 1971, 28, 131.
10. Butcher, R. G. *ibid.* 1972, 32, 171.
11. Bitensky, L., Butcher, R. G., Chayen, J. *in* Lysosomes in Biology and Pathology (edited by J. T. Dingle); vol 3, p. 465. Amsterdam, 1973.