Y Chromosomes and Quinacrine Fluorescence Technique

SIR,—The observation that part of the long arm of the Y chromosome fluoresces brightly with quinacrine mustard[1 2] has led Pearson, Bobrow, and Vosa,[3] using quinacrine hydrochloride, to the discovery that a fluorescent body presumably derived from the Y chromosome is present in the nuclei of epithelial cells in buccal smears, and of cultured lymphocytes, from male subjects. Barlow and Vosa[4] have observed a similar body in a proportion of spermatozoa and Pearson and Bobrow[5] using this technique have shown that it is the short arm of the Y that associates with the X during first meiotic prophase.

Any test for the presence of Y chromosomes in interphase cells is complementary to the sex chromatin test and has potentialities of similar magnitude. Using 0·5% quinacrine (mepacrine hydrochloride *B.P.*) and following the method of Pearson *et al.*[3] I have found this to be a relatively simple technique applicable to material prepared in a variety of ways. In the male a "Y chromatin" fluorescent body can be demonstrated in a number of different cell-types. Thus, in small lymphocytes, which of course are unsuitable for the sex chromatin test, the Y chromatin can easily be seen as a peripherally-situated body separated from the rest of the nucleus by a relatively clear area (Fig. 1). On the basis of the presence

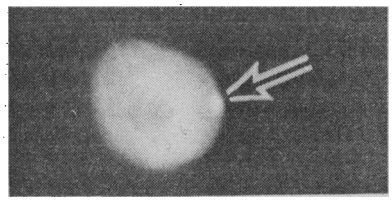

Fig. 1. Small lymphocyte from tonsillar tissue (male, aged 6). × 2,660.

of the body in lymphocytes, fibroblasts, epithelial, or endothelial cells I have been able to "sex" correctly coded smears of blood and sputum as well as smears made from tonsillar tissue removed at operation, squashes of normal and malignant tissue fixed in acetic alcohol, and smears prepared for chromosome studies (by air-drying suspended material on to slides after colchicine and hypotonic pretreatment). Smears fixed by freeze-substitution have also proved suitable, the Y chromatin body often being seen in over 75% of the cells. Material stored for seven years in a domestic deep-freeze unit has not shown any marked diminution in the brightness of the body. As with the sex chromatin test, buccal smears though convenient to obtain are a little less suitable than other materials, especially if the cells are overlaid by bacteria which are of the same order of size as the chromatin body.

Preliminary results have shown that the Y chromatin body is present in the cells of most tumours of males, a similar body not being seen in those of females. The question of the origin of sex chromatin in testicular teratomas being as yet unresolved, particular interest attaches to the presence or absence of Y chromosomes in the karyotypes of these tumours. I have found that the epithelial cells in two malignant teratomas of the testis (patients aged 22 and 57) contained both sex chromatin and Y chromatin bodies (Fig. 2). Unfortunately, it

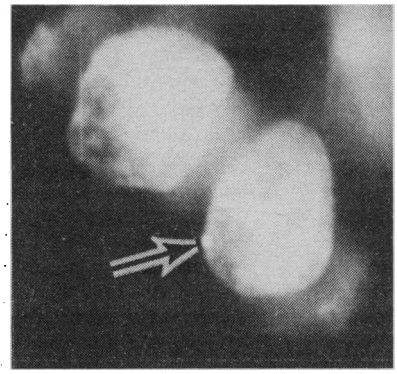

Fig. 2. Group of malignant epithelial cells testicular teratoma (aged 22). × 1,660.

was not possible to stain the cells satisfactorily for sex chromatin after application of the fluorochrome, and it is therefore uncertain whether the same cells contain both bodies. The presence of Y chromosomes as well as sex-chromatin-forming X chromosomes in testicular teratomas would clearly have some implications for the genesis of these tumours; thus, their origin from an X-chromosome-containing haploid cell which underwent chromosomal duplication, as proposed by Tavares,[6] would obviously be ruled out. The presence of a near-triploid modal amount of DNA in the cells of each of the tumours mentioned above is, however, compatible with their origin from a triploid cell with an XXY sex chromosome complement.

A further example of the usefulness of the quinacrine fluorescence technique is provided by a spontaneously-aborted fetus and placenta which, while failing to yield satisfactory chromosome preparations, were found to have a near-triploid DNA content, two sex chromatin bodies in many of the cells, and no Y chromatin body—these together suggesting a 69, XXX karyotype.

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—I am, etc.,

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Priapism and Phenothiazines

SIR,—In an article by Drs. D. Meiraz and J. Fishelovitch[1] discussing priapism and medication with chlorpromazine the authors state their belief that this medication had not been mentioned in this connexion before. However, I would like to draw readers attention to my paper[2] which refers to this topic.

Meiraz and Fishelovitch give details of four cases. One of these patients was diagnosed as suffering from schizophrenia and one from depression. The other two were described as having been treated with chlorpromazine for a "psychological disorder" and "nervousness." The case I described was diagnosed as suffering from schizophrenia.

In considering these five reported cases it becomes apparent that priapism occurring on chlorpromazine therapy is not confined to schizophrenic patients; it affects both young and old patients; it may follow administration of the chlorpromazine either by injection or via the oral route; withdrawal of chlorpromazine or changing medication to a different phenothiazine seems to result in resolution of the condition; and it is not race-specific.

Meiraz and Fishelovitch suggest that the hypotensive effect of the chlorepromazine may be the basis for development of the priapism. The patient to whom I referred had been intensively investigated and his blood pressure taken many times but, so far as I am aware, at no time was he found to be hypotensive. Also it should be noted that a very large number of patients taking chlorpromazine medication are potentially at risk, yet I know of only five reported cases of the condition. Furthermore, other phenothiazines are known to cause hypotension, yet when I checked with the makers of some of these none knew of a single case of priapism occurring in conjunction with administration of their product.

Nevertheless, as Meiraz and Fishelovitch indicate, priapism has been reported in association with medication by other hypotensive agents. I feel more inclined to support the suggestion of these authors that the explanation lies in an idiosyncratic response of the automatic nervous system rather than central hypotension.

I am grateful to Dr. Rosemary Atherton for drawing my attention to the article by Drs. Meiraz and Fishelovitch, and also to May and Baker, Sandoz, and Smith Kline and French who kindly searched their available literature for me.—I am, etc.,

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Urinary Tract Infection

SIR,—Dr. A. J. Wing (26 September, p. 753) highlights some of the problems facing the general practitioner in attempting to assess the significance of symptoms suggesting urinary tract infection encountered on 12 to 20 occasions in every 1,000 consultations (17 patients in a personal series of 1,000 consecutive patients last year). There is evidence[1 2] that an even higher proportion of patients than the 50% suggested by Dr. Wing may have no treatable bacteriological cause for symptoms. Such patients cannot on clinical grounds alone be separated from those who need antibacterial treatment. It follows that the general practitioner may feel obliged to take specimens from all patients with symptoms.

The lack of van collecting facilities, communication problems, and insufficiently clear incentives all combine to make this a

chore unlikely to be undertaken readily by general practitioners. Again, if it were undertaken, the number of specimens would be such as to swamp all available laboratory facilities (possibly something like 17,000 specimens would need to be obtained, transmitted, and examined in the laboratory each day throughout the United Kingdom). The introduction of convenient media for semi-quantitative bacterial counts could help to cut this volume of work by about one third if the new technique allowed for incubation and reading on the practice premises; the positives and doubtful positives only would be transmitted to the laboratory. There is nothing mystical about the technique, and personal experience supports the contention that it is well within the capability of a practice sister or other suitably trained worker. Three requirements must be met for this change in primary medical care to come about:

Equipment: the commercially available media and a simple incubator would need to be readily available to all general practitioners who wish to use this method.

Organization: including the establishing of van collection services, and better communications with laboratories.

Demonstration: that this method in fact raises the quality of medical care.—I am, etc.,

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Misuse of Medical Profession

SIR,—I am writing to support with all my heart the plea[1] that some action be taken over courageous Russians incarcerated for political reasons in mental hospitals. The misuse of the medical profession for the purpose of repression by the State conjures up a horrifying vision, and I call on the British Medical Association to stand up and speak out for its noble principles.—I am, etc.

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REFERENCE

1 *Hospital Times*, 11 September, 1970, p. 6.

Ban on Amphetamines

SIR,—While I share Dr. M. J. Leverton's concern over the theft of stock bottles of amphetamines from the premises of retail chemists (26 September, p. 770) it is far from certain that prescribing in the manner he suggests will contribute to a solution unless he happens to be the only practitioner in the area who prescribes these drugs. Until their prescription becomes a rarity chemists will continue to carry stocks of a convenient size.

As Dr. Leverton says, the patients for whom these preparations are genuinely required are rare. A much more effective solution lies in the restriction of their availability to hospitals, as suggested earlier this year.[1] This has already proved most effective in the case of injection of methylamphetamine, and an extension of such a voluntary arrangement to amphetamines in tablet form would avoid the need for any retail chemist to carry stocks of any size however small.—I am, etc.,

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Serological Diagnosis of Rubella

SIR,—Estimation of IgM haemagglutination-inhibiting antibody after separation by sucrose density centrifugation is an accurate tool both for confirmation and for exclusion of acute rubella in pregnancy. We report elsewhere[1] that IgM antibody was demonstrated in all women examined who had acute rubella in early pregnancy. IgM antibody was absent later than one month after symptoms; in all but one women examined immediately after birth of a congenital rubella infant; and in women who did not have acute rubella. The procedure has been simplified by testing a single gradient fraction known to contain IgM and no IgG.

The method was first evaluated mainly on nonpregnant subjects by Finnish workers[2] and in Dr. Banatvala's laboratory.[3] An important finding from the recent paper of the London group[4] is that in a laboratory which also examines difficult cases referred by other laboratories half of the serological confirmations of acute rubella in pregnancy were made by the detection of rubella-specific IgM, and would have been missed by the usual criterion of rising antibody titres.

For diagnosis possibly implying therapeutic abortion well-standardized laboratory technique of high specificity and sensitivity is mandatory. Among methods used to determine rubella-specific IgM antibody 2-mercaptoethanol sensitivity of whole serum falls short of these standards[1]. Density gradient centrifugation comes nearest to the aim. With immunofluorescence, it appears from the first publications[5-7] that there may be problems with sensitivity or specificity. Cohen *et al.* consider rubella IgM titres of 8 as nonspecific; in acute rubella only two patients out of 13 developed titres higher than 16.[7] Detection of rubella-specific IgM antibody by immunofluorescence now seems to have been considerably improved in Professor Fraser's laboratory,[8 9] and comparative evaluation of the ultracentrifugation and immunofluorescence methods is eagerly awaited.

The ultracentrifugation method in its simplified version[1] is not truly complex, but the requirement for 18 hours of ultracentrifugation is maintained. With present rotors, usually three, a maximum of six specimens including controls if desired can be tested simultaneously. With immunofluorescence more specimens could be handled at smaller expense. However, technical implications are still such that we doubt whether the method will be profitably used by many laboratories other than those which are able to perform gradient centrifugation when indicated.

It may take years before vaccination makes laboratory help in suspected rubella during pregnancy an uncommon request. Since vaccination became available in Belgium more than a year ago, requests have sharply increased in our and other laboratories. We attribute this increase primarily to growing awareness of the rubella problem, generated by information on the vaccine.—I am, etc.,

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Folate and Vitamin B_{12} in Epilepsy

SIR,—Dr. N. S. Gordon's letter (25 July, p. 226) concerning the role of folate and vitamin B_{12} in epilepsy is a timely one, and I feel that his statement of caution regarding the acceptance of a relationship between seizures and folate deficiency should not go unheeded.

Much of the evidence concerning the alleged worsening of seizures and improvement in mental state after folate administration is based on scanty or even solitary[1] case histories. Unfortunately, the uncontrolled administration of folic acid, vitamin B_{12}, or both to a group of patients (27 June, p. 759) without comparison to the effect of placebo is difficult to evaluate. This is even more difficult when the parameters to be measured are as variable as seizure frequency, or improvement is as vague as "more relaxed," "happier," etc.

Two attempts at controlled evaluation of folate in epilepsy[2 3] have failed to show any relationship. In a recent controlled trial of the drug at the Montreal Neurological Institute[4] folic acid was found to have no more effect on seizure frequency than placebo. In fact one patient developed severe status epilepticus on placebo.

This field has been one in which speculation and prediction have expanded beyond all reasonable limits, and the need now is for an entity always more painful to acquire—namely the facts.—I am, etc.,

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