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Fetal chromosome analysis in the Northern Trent region
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Specimens referred from over 40 obstetric practices are sent to a central laboratory. About 1500 specimens per annum are currently processed. Specimens are received the day of, or the day after, amniocentesis. Of specimens referred because of advanced maternal age, 2% exhibited fetal chromosome abnormality of clinical significance. Specimens (493) taken because of a raised maternal serum AFP level yielded four chromosome abnormalities (0.8%) where maternal age was less than 35 years. Two of these four pregnancies had already been terminated because of a high amniotic fluid AFP level. Although currently 93% of pregnancies examined are successfully reported upon, in 12% a repeat amniocentesis is required. Factors outside laboratory procedure, for example, the time of the specimen in transit, influence the frequency of culture failure. Obstetric practice is an important variable. Data do not indicate that chromosome abnormality is disproportionately represented in those specimens where chromosome analysis is unsuccessful.

High resolution chromosome analysis from peripheral blood samples by fluorescent activated chromosome sorting
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A new technique has been developed for high resolution chromosome analysis of lymphocyte chromosomes from short term blood cultures. Chromosome suspensions are made from colchicine-arrested metaphases, lysed with detergent, stained in ethidium bromide, and analysed in a standard FACS II flow cytometer. Flow karyotypes with up to 20 peaks can be obtained with coefficients of variation in the range of 1 to 2%. It is possible to identify the chromosomes which contribute to each peak and the profile is sufficiently reproducible to allow the detection of even comparatively minor variations in individual karyotypes. Before the method can be used to screen for unbalanced chromosome aberrations it is necessary to determine the extent of variation through chromosomal polymorphism. Correlations between cytological and flow karyotypes have been obtained for several different centric heteromorphisms, Y chromosome polymorphisms, and examples of numerical and structural chromosome abnormalities.

Variations in replication pattern and phenotype in two cases of X chromosome inversion
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An inverted X chromosome was identified in two cases with contrasting replication patterns and phenotypes. Case I was a 15-year-old girl with features of Turner's syndrome. A paracentric inversion of the X chromosome, 46,XX,inv(X)(p12;p22), was found in all cells and this chromosome was invariably late labelling. Both parents were normal. Case II was a 9-month-old female referred with coloboma. A pericentric X inversion, 46,XX,inv(X)(p22;q12), was seen in all cells and this abnormality was inherited from a phenotypically normal father. The X chromosome inactivation pattern was random. In most reported cases inverted X chromosomes show random inactivation and a normal phenotype is observed. Non-random inactivation of an inverted X chromosome may therefore be significant in assessing the effect of balanced X chromosome rearrangements.

Nephroblastoma and the 11p—chromosome
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The association between nephroblastoma, eye defects, mental retardation, and a specific chromosome abnormality has previously been reported. Our experience with this association, although necessarily limited, poses many questions relevant to diagnosis, prognosis, and treatment in affected children. Scant attention appears to have been paid to the possibility of specific histological features in these tumours. The relationship of such features to persistent renal blastema is of considerable interest. In infants and children referred for chromosome analysis because of psychomotor retardation or odd facial features and found to have an 11p deletion, continued clinical surveillance throughout childhood would appear mandatory, bearing in mind the possibility of subsequent development of nephroblastoma. Such monitoring could include regular ultrason. Until more information concerning the association emerges it would be of value to karyotype blood of all children with nephroblastoma, as well as the tumour itself. Finally, the prognosis for children with this particular association between tumour and chromosome abnormality remains open to debate. Are extensive chemotherapeutic and radiotherapeutic regimes necessarily advisable, particularly in the very young?
Fragile-X chromosome in brothers with normal intelligence, or the man who might have been a genius?
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An Indian couple were referred for chromosome analysis because the wife had had six pregnancies which all ended in spontaneous abortion. She had a normal female chromosome complement, but the husband was found to have a fragile site on the X chromosome in 15% of G banded metaphases. Cultures were grown in TC 199 supplemented with either 5 or 20% donor calf serum. The fragile site appeared to be identical to that found in subjects with typical X-linked mental retardation. The patient is aged 42 with somewhat eunuchoid proportions and a testicular volume of 20 ml. He is of normal intelligence and works as an office clerk. The only relative available for study is an older brother who has four healthy daughters who are said to be doing well at school. This man was also found to have the X-chromosome fragile site in approximately 9% of his metaphases. He, too, is of normal intelligence and works as a telephone engineer. The implications of these unexpected findings will be discussed.

Studies on the mixed cell population derived from testis tubules
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The various cellular stages of the spermatogenic cycle can be categorised from the array of cells observed in the mixed cell population derived from testis tubules. Cell category frequencies were studied in a series of infertile males. Considerable variation existed between subjects. Significant relationships were found between some cell categories in the series. One such significant relationship suggested that one particular category was an important estimator of the activity of the germinal epithelium. The ratios between the other categories were affected by the presence of this varying category. The ratios were used to identify points of lesion in the spermatogenic cycle. The results suggested that some ratios within the cycle were remarkably constant despite the heterogeneity of the population sampled. The implication of these results is that the process of spermatogonial proliferation and meiotic division is not easily disrupted.

Abnormalities of the umbilical cord or membranes leading to raised amniotic AFP
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Fetal defect resulting from abnormal development or position of the umbilical cord or membranes may be a relatively common cause of raised amniotic AFP. Where this aetiology is overlooked or misdiagnosed inaccurate recurrence risks may be given for the fetal defects seen. Six examples are described, five of which were detected by serum AFP screening. They were from 1000 consecutive amniocenteses, 200 of which were for high serum AFP. Aberrant tissue bands were present in three cases, where fetal defects seen included apparent anencephaly, gastroschisis, amputations, and constrictions. In the other three cases cord abnormalities were involved. All six cases were identified as non-NTD abnormalities by a combined use of amniotic AFP and AChE. The interpretation of these tests, the origin of the amniotic ACP, and the recurrence risks in the individual cases are discussed.

Hypomelanosis of Ito: a neurocutaneous syndrome
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Hypomelanosis of Ito is a neurocutaneous syndrome comprising hypopigmented areas of skin in whom, skin streaks, and patches. The distribution of hypopigmented skin lesions resembles that of the hyperpigmented lesions in incontinentia pigmenti, and for this reason the condition has also been called incontinentia pigmenti achromians, but the two disorders are unrelated. Commonly, other systems are involved, especially the brain. The evidence for any sort of genetic aetiology is inconclusive, though parental consanguinity has been noted on three occasions. The present case is the fourth child of two Pakistani parents who are first cousins. Striking depigmented areas, which stop at the midline anteriorly, were evident at birth and onset of intractable seizures was in the first few hours of life. Computed axial tomography shows cerebral atrophy, and a ventricular septal defect that was present in infancy has closed spontaneously.

A clinical and genetic study of Hunter’s syndrome
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Hunter’s syndrome is the X-linked form of mucopolysaccharidosis. Information has been obtained on 66 definite (23 isolated and 43 familial from 14 kindreds) and 22 probable (14 isolated and eight familial from three kindreds) cases, of whom 44 are still alive. The patients fell into two clear groups, mild and severe, on the basis of intellectual performance, and the disorder ‘bred true’ in all families with one possible exception, to be discussed. In the severe group of 51 cases, the average age of onset was 30 months and average age of death 12 years. These children achieved peak performance level at 4 to 6 years and then regressed losing all skills by 10 years in most cases. In the mild group of 30 cases, the average age at onset was 50 months and average age at death 22 years. These patients maintained relatively normal intellectual performance. Lod score analysis indicates that the Hunter and Xq loci are not closely linked.
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Carrier detection in Hunter's syndrome (mucopolysaccharidosis type II)
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The Hunter syndrome is a result of a defect in the enzyme iduronate 2-sulphate sulphatase and, because of its X linked recessive inheritance, carrier detection is of special importance. To achieve this, iduronate 2-sulphate sulphatase levels have been measured in hair roots and sera of obligatory and possible carriers. All of 14 obligatory carriers showed clear evidence of a double population of hair bulbs as did 15 of 32 possible carriers with a high and defined genetic risk. However, four of the 32 possible carriers could not be classified with confidence either as normal or as carriers. Using a more sensitive serum assay than previously used by I Liebaers and E F Neufeld (Pediatr Res 1976;10:733), obligatory and possible carriers (35 in total) had on average half the mean serum level of iduronate 2-sulphate sulphatase activity, but the range of enzyme activities of obligatory carriers overlapped those of the normal range. By combining the distribution of serum enzyme levels of obligatory carriers and normal controls, a series of likelihood ratios has been constructed which shows that the serum assay also has considerable predictive value in carrier detection when used in conjunction with the genetic risk.

Reversal of clinical features of Hurler's disease after treatment by bone marrow transplant
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A bone marrow transplant was given to a one-year-old boy with Hurler’s disease in an attempt to replace the deficient enzyme alpha-L-iduronidase (iduronidase). Before treatment he had typical coarse facies, hepatosplenomegaly, corneal clouding, dystrophy multiplex with lumbar gibbus, severe deficiency of leucocyte iduronidase, and markedly raised urinary dermatan and heparan sulphates. After failure of engraftment of his paternal marrow he was grafted with maternal marrow. Evidence of engraftment included: 88% female bone marrow cells by day +14; 100% female peripheral lymphocytes on days +70 and +176; leucocyte iduronidase activities started to rise by day +14, reaching heterozygote levels on day +37, where they have been maintained until the present (8 months after the graft). Iduronidase activity appeared in the serum and urine and was accompanied by considerable degradation of glycosaminoglycans excreted in the urine. Graft versus host disease developed, but was partially controlled by steroids. Clinically, the hepatosplenomegaly has disappeared, the corneal clouding has cleared, and development is progressing normally.

Problems associated with the assessment of thiamine responsiveness in maple syrup urine disease
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A one-year-old child with maple syrup urine disease exhibited 12% residual leucine oxidative activity in cultured fibroblasts. In vitro studies suggested increased leucine oxidation in response to long term incubation of the patient’s fibroblasts with thiamine. The patient was subjected to an oral therapeutic trial of thiamine, initially at 100 mg/day followed by stepwise increases of 200 and 300 mg/day. Blood samples were taken during the trial and the rate of oxidation of leucine by whole blood determined. After a lag period of approximately 3 weeks there was a steady rise until at 6 weeks the activity was twice the pretreatment level as compared to a control subject sampled at the same time. The next determinations at 21 weeks and 35 weeks showed values below the pretreatment level. Although there was no dramatic immediate clinical improvement the frequency of acute crises during home management decreased.

A genetic study of cryptorchidism
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Although undescended testis occurs in several Mendelian dysmorphic syndromes, the cause of isolated cryptorchidism per se is unknown. Individual family reports and larger epidemiological studies suggest that hereditary factors may be involved. Family studies were sought from the parents of 77 boys treated for this condition, of whom 51 agreed to co-operate. The incidence in first and second degree male relatives of the probands was found to be 6.5% and 5.0%, respectively, with values of 3.9%, 9.75%, 4.0%, and 6.6% obtained for fathers, brothers, paternal uncles, and maternal uncles, respectively. No clear pattern of Mendelian inheritance emerged in pedigree analysis. Laterality did not breed true. Possibly, cryptorchidism is multifactorially inherited or alternatively genetic heterogeneity may be involved as in animals. The relatively high incidence in sibs prompts us to urge doctors active in the management of this condition to pay attention to the rest of the family.

Antenatal diagnosis of neural tube defect in the Northern Trent region
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Antenatal diagnosis of NTD by MS-AFP screening was initiated in the Northern Trent Region in 1977 with a centralised laboratory service to six major antenatal units and family practitioners in five health districts. The service has been gradually extended during the subsequent 3 years to give a full sub-regional service to nine health districts in 1980. The centrally financed service is open to...
both hospital and family practitioner obstetric services. Blood samples taken at antenatal examination are sent by van or mail to the central laboratory for MS-AFP assay. All results over the discriminant limit are communicated verbally by the laboratory director to the requesting clinician with a recommendation as to appropriate action. Subsequent amniotic fluid samples are sent to the same laboratory for AF-AFP assay and secretor acetyl choline esterase isoenzyme identification. In the 4 years 1977 to 1980 the laboratory has screened 64,574 pregnancies with the identification of 115 cases of open neural tube defect at the cost of five normal fetuses. The results of the screening programme are under continual audit with follow-up of outcome in all cases in which a raised MS-AFP is recorded.

A double randomised controlled trial for preconceptional folic acid supplementation for the prevention of recurrence of neural tube defects in high risk pregnancies

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Out of 905 women in South Wales who had a previous neural tube defect, who were asked to co-operate in the trial by taking a tablet (containing 2 mg of folic acid or placebo) twice per day from the time that a pregnancy was being attempted, only 51 women assigned to the placebo group and 60 to the folic acid supplementation group could be included between 1969 and 1974. Of those on folic acid supplementation, 16 were found not to have taken the tablets during the first trimester, leaving 44 'protected' women, none of whom had a recurrence. The 67 women (including the 16 non-compliers) had six recurrences. Bearing in mind the role of an inadequate diet in the first trimester in the genesis of NTD the ten folic acid protected women on poor diets were free from recurrences, while the 17 non-protected had six recurrences. The protective effect of folic acid is a statistically significant one. It is concluded that if these findings are confirmed in a larger (multi-centre) trial folic acid supplementation would be an effective method of primary prevention for all high risk pregnancies, and probably a suitable, sufficiently cheap, and scientifically ethical method of protecting all the pregnancies in the high incidence community, reducing the need for invasive prenatal diagnostic tests and selective abortion and confining serum AFP screening to the unplanned pregnancies only.

Genetic aspects of achalasia

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Achalasia is a neuromuscular disease, the pathogenesis of which is still not clear. Environmental agents such as *Trypanosoma cruzi* infection (Chagas disease) can produce achalasia, but genetic factors must also play a role. About 24 reports of familial occurrence are known; in most families only sibs are affected with onset in childhood. More than one generation have been affected in six families all with onset in adulthood. We present here details of a family with 15 patients in five generations. The following preliminary classification of achalasia can be made. (A) Environmental (eg Chagas disease). (B) Genetic (1) Infantile type (autosomal recessive). (2) Adult type (autosomal recessive). (3) Adult type (autosomal dominant). (4) Adult type (multifactorial?). (5) Achalasia as a part of a syndrome.

Fibroblast alkaline phosphatase in fibrodysplasia ossificans progressiva

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Skin fibroblasts were grown under strict in vitro conditions from 27 normal subjects and six patients with fibrodysplasia ossificans progressiva (FOP). Alkaline phosphatase activity was assayed with a fluorimetric substrate, and acid phosphatase was used as a reference enzyme. In 53 strains from the normal subjects the level of alkaline phosphatase activity ranged from 7.3 to 59.8 nmol 4 MU/mg protein/hour at 37°C, and these levels were inversely proportional to the age of the donor (regression coefficient = -0.23, SE=0.074). In 21 strains from the FOP patients the level of activity ranged from 8.2 to 41 nmol 4 MU/mg protein/hour at 37°C, and these levels did not differ significantly from age matched controls. Skin fibroblast alkaline phosphatase was also examined by isoelectric focusing. With this technique only a single band was apparent in both the FOP and normal subjects and the band had the same isoelectric point in each case. Thus skin fibroblasts in FOP appear to have normal qualitative and quantitative regulation of alkaline phosphatase, which may suggest that the defect in FOP lies in the extrinsic control of the cells which form the abnormal bone.

Heterogeneity in osteogenesis imperfecta: a review of 125 South African patients

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Osteogenesis imperfecta (OI) is a well-known inherited connective tissue disorder. The manifestations, which include skeletal fragility, limb bowing, growth impairment, alteration of scleral colour, dentinogenesis imperfecta, and a propensity to late onset deafness, are very variable. It is likely that OI is heterogeneous and four major types have recently been delineated. In an attempt to validate this new classification, 125 South African patients with OI from 65 separate families have been examined. These patients fell into the following categories:

Type I: Mild fracturing, blue sclerae, deafness, AD inheritance.

60 patients in 27 families:
With dentinogenesis imperfecta, eight patients in four families.

Without dentinogenesis imperfecta, 52 patients in 23 families.

Type II: Lethal in perinatal period. AR inheritance, 12 sporadic patients.

Type III: Severe dwarfism, fracturing, and limb bowing, white sclerae, AR inheritance, 16 patients in 11 families.

Type IV: Heterogeneous unclassifiable forms of OI, 17 patients in 14 families.

Type V: Minimal bone fragility, blue sclerae, wormian bones, dentinogenesis imperfecta, AD inheritance, 20 patients in a single family.

We conclude that the limits of syndromic resolution on a clinical, radiographic, and genetic basis have now been reached.