**Editorial**

Lateral reading 8

HONEYBEES, ENERGY SUPPLY, AND BIRTH DEFECTS

Honeybees are susceptible to poisoning by mannose. Freinkel *et al* (*N Engl J Med* 1984;310:223-30) were interested in the reason for birth defects in diabetic pregnancies. They therefore tested the effect of high glucose concentrations on the 9 to 11 day old rat embryo grown in vitro. Mannose was one of the five hexoses chosen as controls, but surprisingly it proved a more potent teratogen than glucose. The implications of this in relation to glycolysis are discussed and the leader writer thinks that these findings should focus the attention of teratologists upon the supply of energy in the embryo and upon the many different ways in which it might be compromised. For example, birth defects might be caused by mutations affecting the enzymes controlling the glycolytic pathway enzymes. The possibility is raised that the type of genetic lesion with which we are familiar in inborn errors of metabolism, causing effects such as mental deficiency, may give rise to different effects such as malformations when they occur in the developing embryo, the energy available for cell division being restricted. For example, physical malformations have been described in glutaricacidemia as well as the near-lethal acidosis. Multiple physical defects were also found in a baby with β-hydroxyisobutyryl CoA deacylase deficiency. One paper not mentioned in the leader gave some support to these views using the rat embryo (*Garnham et al. *Diabetologia* 1983;25:291-5). It shows how alterations in the glucose levels may give rise to neural tube defects. A second paper (Sheehan *et al*, in press) describes how similar defects are produced by ketone bodies. It seems that the knowledge that babies born to diabetic mothers are much more likely to have birth defects is well worth pursuing from metabolic aspects.


A leader in the *BMJ* suggests that there may now be as many as 1500 cases of Wilson's disease in Britain, a considerable proportion of which are unreco gnised. The leader continues: "Untreated, Wilson's disease is always progressive and fatal. Treated, most patients have a normal life of normal length (unless terminally ill with liver or neurological damage when the diagnosis is made). This contrast is so dramatic, and the clinical picture so variable, that anyone between the ages of 5 and 50 with unexplained liver disease, enlarged liver and spleen, hypersplenism, or attacks of jaundice (the more frequent initial manifestations) should be considered to have Wilson's disease until otherwise proved. Similar suspicion should be aroused by signs of brain damage, tremor, clumsiness, ataxia, rigidity, failure at school, epilepsy, speech disorder, or dementia. Perhaps a fifth of patients present with a purely psychiatric illness, and a few with renal or bone disease. All these are doomed to die in coma, bleeding, mute, immobile, or demented-unless the correct diagnosis is made and treatment given." Treatment is now by decoppering with penicillamine. This needs to be continued throughout life but is curative, though the clinical improvement is sometimes slow. If penicillamine cannot be tolerated (side effects are not uncommon) the most effective therapy is triethylene tetramine dihydrochloride. This information is more for general physicians and psychiatrists than medical geneticists, but it deserves the widest publicity.


Glycogen storage disease type IIa is a fatal disease controlled by an autosomal recessive gene. Histologically it is characterised by the presence of intracellular vacuoles full of glycogen which are found in most tissues. The authors carried out electron microscopical examinations on uncultured amniotic fluid cells from 26 women whose fetuses were at risk for the disease and eight normally pregnant women used as controls. It was found that in six of the 26 high risk patients the specific vacuoles were present and in each case the child was found to be affected at birth. The other 20 were normal, as were the controls, consistent with the method of inheritance. The material was obtained at amniocentesis at 15 to 18 weeks and electron microscopy results were available in 3 to 6 days. On the other hand it took 3 to 6 weeks to make the diagnosis by enzymatic analysis of the cultured cells. The fact that
Prenatal diagnosis can be made so much earlier by electron microscopy is of great help in considering the question of therapeutic abortions in this disease.

MATERNAL SERUM ALPHAFETOPROTEIN MEASUREMENT: A SCREENING TEST FOR DOWN SYNDROME (Cuckle HS, et al. Lancet 1984;i:926)

Drs Howard S Cuckle, Nicholas J Wald, and Richard H Lindenbaum summarise their paper as follows.

"The median maternal serum alphafetoprotein (AFP) level at 14 to 20 weeks' gestation in 61 pregnancies associated with Down syndrome was 0.72 multiples of the median (MoM) value for a series of 36 652 singleton pregnancies unaffected by Down syndrome or neural tube defect—a statistically significant reduction. The difference is great enough to form the basis of a screening test. By selecting for amniocentesis women with serum AFP levels ≤0.5 MoM at 14 to 20 weeks' gestation (excluding any of these that ultrasound cephalometry shows to have been due to the overestimation of gestational age), 21% of pregnancies with Down syndrome would be identified as well as 5% of unaffected pregnancies. If amniocentesis were offered to all women aged 38 years or more and, in addition, to younger women with serum AFP below specified maternal age dependent cut off levels (≤1.0 MoM at 37 years, ≤0.9 at 36, ≤0.8 at 35, ≤0.7 at 34, ≤0.6 at 32 to 33, ≤0.5 at 25 to 31), 40% of pregnancies with Down syndrome and 6.8% unaffected pregnancies would be selected. The screening policy we recommend would require greater clinical and cytogenetic resources, but these costs would need to be considered in the light of the reduced costs of special care for children with Down syndrome."

What do readers think about this? Correspondence to the Journal invited.

RECESSIVE MUTATION IN AETIOLOGY OF WILMS' TUMOUR (Leader. Nature 1984;309:111–2)

"Much has been written and published in recent months about the cellular genes whose activation by retroviruses, mutation and chromosomal rearrangements seems to convert them into dominantly acting oncogenes involved in tumour initiation or promotion. Less has been heard of tumours which seem to be the result of the deletion or inactivation of both copies of a wild-type genetic locus and therefore to be the result of a recessive mutation. The only specific recessively acting mutation to have been studied in any detail until now is that associated with rearrangements of chromosome 13 in the childhood tumour retinoblastoma. But in the same issue of Nature there are reports from four independent laboratories of genetic changes in Wilms' tumour resembling those documented in retinoblastoma.

Like retinoblastoma, Wilms' tumour occurs in childhood in both inherited and spontaneous (sporadic) forms. In a small percentage of both forms, a specific deletion of band p13 of chromosome 11 has been observed. This deletion provides a clue as to which chromosomal region might be involved in Wilms' tumour and, along with the hypothesis of Knudsen that at least two mutational events are necessary for tumour formation, provides a basis for the investigations. Thus if a gene on one of the pair of chromosome 11s is deleted or inactivated, a second mutational event on the homologous chromosome is postulated to be necessary for tumour formation. Since the nature and precise location of the inactivated gene is unknown in both retinoblastoma and Wilms' tumour, small structural changes cannot be looked for, and it has therefore been necessary to focus on evidence of gross chromosomal changes within the tumours which might result in the deletion or rearrangement of the second locus. All four papers in this issue show that in a large proportion of Wilms' tumours such changes can be seen.

Several different mechanisms could account for inactivation or deletion of the homologous locus. Among the events involving whole chromosomes that can be detected without knowing which locus to examine are (a) loss of a whole chromosome so that only one homologue remains (hemizygosity), (b) loss of one chromosome and reduplication of the other (homozygosity), (c) mitotic recombination (crossing over between chromosome homologues in somatic cells during mitosis) so that the part of the chromosome containing the mutation becomes homozygous, and (d) translocations leading to deletion or inactivation of the locus.

The methods used to investigate mechanisms a, b, and c make use of the naturally occurring genetic variations found between chromosomes 11 homologues. These are detected with DNA probes that identify differences in sites of cutting of various restriction enzymes (restriction fragment length polymorphisms). In normal tissue, a certain number of sites on the homologues will differ, the chromosomes being heterozygous at these sites. The new papers report a comparison between normal tissue and tumour tissue of Wilms' tumour patients with respect to such heterozygosity. The DNA is cut with appropriate restriction enzymes and Southern blot analysis performed with a variety of probes, all directed to the tip of the short arm of chromosome 11. The probes recognise sequences flanking the insulin gene, β globin gene, parathyroid hormone gene, the cellular homologue of the transforming gene of
Harvey murine sarcoma virus (c-Ha-ras), and a random human genomic sequence. The salient observation from all the studies is that in cases where normal tissue is heterozygous, the tumour is often homozygous—the predicted result of loss of one chromosome or recombinational events as in mechanisms a, b, and c. The end result is loss of the functional ‘Wilms’ locus’ from both chromosomes.

The major conclusions from this work are, first, that somatic mutational events are at least as likely to be caused by gross chromosomal changes as by more site-specific events; and second, that recessive mutations may be an important contributing factor to oncogenesis. The fact that both retinoblastoma and Wilms’ tumour are embryonic tumours, with a significant genetic component, may or may not be significant. Other categories of tumours need to be studied in a similar fashion. Obvious candidates are other childhood tumours, such as neuroblastoma; inherited tumours of adults, such as medullary thyroid carcinoma and familial polyposis coli; and sporadic tumours with later age of onset, such as colonic adenocarcinomas. In none of these cases is it obvious which chromosomes to examine. Chromosome preparations from solid tumours are notoriously difficult to analyse and in few cases other than retinoblastoma and Wilms’ tumour has a very specific alteration been seen. At present, therefore, either clues must come from the inconsistent, but perhaps significant, chromosome changes in these tumours, or enough probes must be used to cover all the chromosomes. Once the location of any recessive mutations is found, attempts to identify the normal gene products by molecular genetic analysis will follow. In the case of inherited tumours, genetic counselling will be greatly helped by the ability to determine which parental chromosome carries the mutant allele.”

**SINGLE BASE DELETION IN THE VASOPRESSIN GENE IS THE CAUSE OF DIABETES INSIDIPUS IN BRATTLEBORO RATS** (Schmale H, Richter D. *Nature* 1984;308:705–9)

In man the basis of treatment for hypophysial diabetes insipidus is vasopressin or one of its analogues and the same is true of rats with the Brattleboro mutant. These animals lack both the hormone vasopressin and its neurophysin carrier. On the other hand the production of oxytocin and its neurophysin carrier remain intact. By using restriction enzyme techniques on normal and mutant rats it has now been possible to work out the molecular basis of the abnormality. Thus, in the Brattleboro rats the defect in the vasopressin gene is the result of a single deletion in the sequence encoding the neurophysin and gives rise to an open reading frame. This predicts a hormone precursor having a different C terminus. The details are beyond me but the findings seem highly relevant to the disease in man, a fact which, interestingly, is not mentioned in the paper.


That there is probably a link between the central nervous system and the immune system is easily accepted. The doubt is whether enough is yet known to sustain people’s hopes of explanation. Can it be that a person’s state of mind, affected, say, by grief, may alter the quality of his immune system? John Maddox discusses the evidence in an interesting *Nature* leader. Before a lecture I mentioned the article to a colleague and he told me that he had heard that in the mouse the antibody response to a standard injected antigen was completely inhibited if a cat sat on the top of the mouse’s cage. Is this true? If so, geneticists may have to consider the emotions when penetration is incomplete.


If a women has an affected father she is an obligatory carrier and 50% of her sons will be affected. Molecular genetics has improved this assessment by using the DNA probe L1.28 which corresponds to a small fragment of the human X chromosome. L1.28 has two distinguishable variants, A1 and A2, and one or other allele will almost certainly be inherited together with the XLRP gene. Bhattacharya et al. were able to establish the linkage of L1.28 with the retinitis pigmentosa gene by tracing the inheritance of both the disease and the A1 and A2 variants through five different families. The L1.28 marker makes it possible to distinguish fetuses bearing the normal X chromosome of the mother from those bearing the defective one, provided that the mother is heterozygous for the marker. This is because, as Miranda Robertson writes in the leader, “if her father’s X chromosome bears the A1 variant she can be certain that any male fetus inheriting A1 will also inherit retinitis pigmentosa, and conversely if he inherits A2 he will be normal. But if she is homozygous—that is she has A1 on both X chromosomes—there is no way of distinguishing a normal male from an affected one before birth. Given the frequency of the two variants in the general population, only 40% of women will be heterozygous “.

Although the causes of renal agenesis and dysgenesis are unknown there are probably genetic factors. However, the point of this paper is purely an empirical one, namely the risks to near relatives of babies born with one or other of the kidney conditions. A total of 71 parents and 40 sibs of 41 index patients were evaluated by ultrasound for genitourinary abnormalities. “Nine percent (10 of 111) had asymptomatic renal malformations, most often unilateral renal agenesis (4·5 percent—a frequency that was significantly higher than the frequency of 0·3 percent among 682 adults [p < 0·004]).” Ultrasound screening is therefore recommended for parents and sibs of patients with the renal lesions.

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