

at a mean age at menarche of 12.4–13.5 years. In many developing countries, the menarchal age is much higher, and probably still declining.

It is reasonable to see this secular trend as correlated with the general fact that children in developing countries have been getting progressively taller and heavier at any given age, presumably due mainly to improved nutrition. In the west, men now reach their maximum height at age 17–18, whereas 50 years ago it was not attained until around age 26. In particular, Frisch and her collaborators have studied the correlation between age at menarche and body weight. They concluded that a minimal level of stored, easily metabolisable energy (in the form of body fat) was necessary for the commencement of menstruation, and assigned a critical body weight of 48 kg at which menarche would typically occur.

As reviewed by Short, these ideas and data remain the subject of debate. Although there are many studies of statistical correlations between age of menarche and nutritional status, there has been little progress in elucidating the specific physiological mechanisms that trigger the onset of puberty. There is, as yet, no easy way of telling when the pubertal waves of ovarian follicular development begin.

Corresponding information about demographic and secular changes in the age of onset of puberty in males is very scant. But this information is somewhat less important in determining a population's net fertility rates; female reproduction is the main rate-limiting process.

Adolescent infertility

In most primitive societies, sexual intercourse begins about the time of puberty and is usually not accompanied by contraceptive precautions. Yet the first pregnancy does not occur for several years. The reason for this adolescent infertility seems to be that ovulation does not typically commence until a few years after the onset of menstruation.

Reviewing anthropological data on the !Kung hunter-gatherers of the Kalahari desert, Kolata (*Science* **185**, 932; 1974) cites 15½ as the average age of menarche and marriage, and 19½ as the average age for producing the first child. In chimpanzees in the wild, 1 to 2.5 years may elapse between menarche and the first conception. Studies based on basal body temperature records of German girls suggest that 60% of menstrual cycles are anovulatory in the 12–14 age group, falling to 45% in the 15–17 group, and 25% in the 18–20 group; maximum fertility is attained around age 26–30 with only 5% cycles anovulatory

Triple overlapping genes

from Maria Szekely

A new sequencing technique and new sequencing results again from Cambridge: this time it is coliphage G4 which illustrates some new tricks a viral genome is capable of in order to compress more information into a small DNA molecule. G4 is a small virus, with a single-stranded circular DNA genome, closely related to Φ X174, the complete primary structure of which has been recently established by Sanger's group (see Sanger *et al.*, *Nature* **265**, 687; 1977; *News and Views* **265**, 685; 1977). Two pairs of overlapping genes have been discovered already in the Φ X174 genome and the same structures exist also in G4 DNA. In addition, however, a third region of overlaps has been found in G4 by Show and coworkers (this issue of *Nature*, page 510). This region shows an even greater efficiency in the use of a single DNA stretch. A tenth viral protein, protein K, which has been detected in relatively large amounts in lysates of G4-infected cells, is coded for by a stretch of DNA which overlaps along its whole length with other genes: its 5' proximal part with the last 86 nucleotides of the gene coding for protein A and its 3' proximal part with the first 89 nucleotides of the gene coding for protein C. At two sites the coding capacity of the DNA is used in all three reading frames. In a TGATG sequence, A is recognised simultaneously as part of the ATG initiation codon of gene K, as part of the TGA termination codon of gene B and as part of the GAT codon for aspartic acid in the sequence of gene A. Similarly in a AAATGAG sequence, the four nucleotides ATGA are recognised in three different reading frames: as part of the codons for Asn and Glu in gene K in the frame AAT GAG; as part of the codon for the last amino acid and the termination codon of gene A in the frame AAA TGA; and as the initiation codon of gene C followed by the beginning of the next, arginine, codon in the frame ATG AGG.

The DNAs of the phages Φ X174 and G4 show a high degree of homology; a gene for a similar protein K could be present in the same position in the Φ X174 genome also. Some genetic evidence has been obtained for the existence of this protein, but it has not been detected

so far in Φ X-infected cells.

These new results have been obtained by a new sequencing technique. Sanger, Nicklen and Coulson recently published a new DNA sequencing method (*Proc. natn. Acad. Sci. U.S.A.* **74**, 5463, 1977), based on similar principles to the plus and minus technique (Sanger & Coulson *J. molec. Biol.* **94**, 441; 1976) which was used in the determination of the nucleotide sequence of Φ X DNA. This new variation is however even quicker, simpler and more accurate than the plus and minus technique.

In the new technique also, a complementary copy is synthesised from a single-stranded DNA template. A random mixture of different length fragments, all ending at a particular base, is obtained using specific chain-terminating inhibitors. These are analogues of nucleoside triphosphates, which have no accessible 3' hydroxyl group. These analogues can be incorporated into the polynucleotide chain through their 5' phosphates but are unable to form further phosphodiester bonds and thus stop further elongation of the chain. By choosing an appropriate ratio of normal nucleoside triphosphate to its chain-terminating analogue, only partial incorporation of the latter will occur and a random mixture of cDNA fragments will be produced which terminate at the different positions at which this particular nucleotide is present. The lengths of the fragments correspond to the different positions of this nucleotide in the DNA sequence.

Four mixtures of fragments can be prepared in the presence of the analogues of the four nucleoside triphosphates. Fractionation according to size will thus reveal the positions of all four nucleotides along the DNA sequence. In this respect these mixtures are similar to the 'minus' mixtures used in the plus and minus technique and the interpretation of the results can be carried out in a similar way. The cDNA fragments in the four mixtures are fractionated by polyacrylamide gel electrophoresis, and from the length of the fragments in the different mixtures, the sequence of the cDNA can be directly read off the gel.

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(Doring *J. Reprod. Fert. Suppl.* **6**, 77; 1969).

Such little evidence as is available

suggests the length of this period of adolescent infertility is roughly independent of the age at menarche. Thus