Technique

The method makes use of the buffer described by Gocke and Howe.[1] This is used for both the electrophoresis tank and for preparation of the agarose gel. The agarose gel is prepared as follows:

0·05 *M* 'Veronal' (sodium barbitone) adjusted to pH 8·2 with 0·1 *N* hydrochloric acid.
0·85% agarose gel is prepared in this buffer.

A pre-cleaned $1\frac{1}{2} \times 3$ in. slide is coated with 6 ml. of molten agarose. Wells 3 mm. in diameter and 5 mm. apart are cut so that there are three double rows of wells, with five pairs in each row (with this up to 15 tests can be performed on each slide). Antiserum to H.A.A. which has been shown to give a reaction of identity with a known specific antigen/antibody system [2] is placed in the anode well, and the serum under test for antigen is placed in the cathode wells. Each slide is then subjected to a potential of 150 volts for ninety minutes at room temperature and the results are read. The slide is now placed at +4°C overnight and is read again. No test shown to be negative after ninety minutes has become positive later. A control positive antigen/antibody system is always included in test runs.

Case-record

A 44-year-old woman was known to be dying from a primary cerebral tumour. The renal transplantation team was informed, and within two hours it was known that she was H.A.A.-free; a lymphocyte-antigen type was also obtained. After her death, which occurred suddenly, 2 units of blood were cross-matched for each of the prospective recipients and a sample of each unit was also tested for the presence of H.A.A. Within two hours the blood was available and known to be both compatible and H.A.A.-free. One recipient required a unit of blood during the operation; no blood-transfusion was necessary in the other case.

Discussion

Until the present test became available the only safeguard, so far as blood for transfusion was concerned, was to use blood from "safe" donors—that is, regular donors thought never to have transmitted hepatitis. This policy is being continued, but we now have an additional check in the direct rapid test for H.A.A.

This test offers a further considerable safeguard to the patients and staff of an organ transplantation unit. The test should have other uses: for instance, we have been able to screen a patient with acute renal failure who required urgent hæmodialysis.

REFERENCES

1. Gocke, D. J., Howe, C. *J. Immun.* 1970, **104**, 1031.
2. Stratton, F. Personal communication.

Hypothesis

EFFECT OF Y CHROMOSOME ON FETAL GROWTH-RATE

CHRISTOPHER OUNSTED
Human Development Research Unit, Park Hospital for Children, Headington, Oxford

MARGARET OUNSTED
Nuffield Department of Obstetrics and Gynæcology, University of Oxford, and Maternity Home, Radcliffe Infirmary, Oxford

Summary An earlier hypothesis, that the greater the antigenic differences between mother and conceptus the greater is the fetal growth-rate, is here extended to explain sex differences in fetal growth-rate in terms of the antigenic disparity created by the presence of a Y chromosome. This effect would be expected in the whole conceptus, and some evidence for this hypothesis can be found in the relative birth-weights of females in mixed-sex multiple pregnancies.

INTRODUCTION

ELSEWHERE [1-3] we have made theoretical proposals concerning factors which control the growth-rate of the human fetus. There is now considerable evidence to support the notion that in some women slow intrauterine growth is determined by a single constraining factor of large and constant effect; these women regularly bear growth-retarded infants.[4] It seems likely that the level at which this maternal regulating mechanism is set is not wholly dependent on the maternal genotype. Its limits may in part be determined by the degree of constraint imposed upon the mother when she herself was a fetus.[5]

Accelerated fetal growth seems to be determined by a number of additive factors. In our series, although the mothers of growth-retarded babies did not differ from controls, the mothers of growth-accelerated babies were older, taller, and heavier than controls.[6] Experimental work on the velocity of placental and fetal growth in mice [7] has shown that immunological factors are involved—the greater the antigenic difference between mother and conceptus the faster the placenta and the fetus grow. We have suggested that antigenic dissimilarity between the human mother and her conceptus may contribute to the enhancement of fetal growth-rate. An orthogonal hypothesis was proposed. We now suggest that such a hypothesis would also explain the difference in fetal growth-rate between the two sexes.

PREDICTIONS

At the sixth week after conception, in a male fetus, the primitive gonads begin to differentiate to produce the male sexual organs. In a female fetus, the only demonstration of her sex is the absence of such differentiation at this time.[8] Femaleness seems to be produced by the lack of a Y chromosome rather than the presence of another X. Information from the Y chromosome is positive, thus, although it is smaller than the X, it produces greater antigenic variety; and boys are, therefore, antigenically more dissimilar from their mothers than are girls. Likewise, a conceptus formed from two zygotes has greater antigenic variety, and therefore would be antigenically more dissimilar from the mother than would a conceptus formed from one zygote.

If antigenic dissimilarity between mother and conceptus enhances fetal growth rate, then (provided

the length of gestation is held constant) we can predict that:

(1) All-male sibships should grow faster than all-female sibships of the same zygosity.
(2) Dizygous twins should grow faster than monozygous twins of the same sex.
(3) Girls in mixed sibships should grow faster than girls in all-female dizygous sibships.
(4) The presence of one fetus carrying the Y chromosome would enhance the growth of the whole conceptus, including that of a female twin.

EVIDENCE

Information is not yet available on the birth-weights of a large unselected series of twins in whom sex and zygosity have been carefully established. However, McKeown and Record's data [9] provide some pointers.

The differences in birth-weight between the sexes diminishes as litter size increases from 0·26 lb. for singletons, to 0·10 lb. for twins, to 0·04 lb. for triplets (same-sex sibships):

Type of Litter	*Mean Birth-weight (lb.)*		*Difference*
	M	*F*	*(lb.)*
Singleton	7·57	7·31	0·26
Mixed-sex twins	5·65	5·27	0·38
Same-sex twins	5·22	5·12	0·10
MFF triplets	4·22	3·94	0·28
MMF triplets	4·01	4·19	0·18
Same-sex triplets	3·92	3·88	0·04

Mean lengths of gestation hardly differed, between the sexes, for singletons and twins, but all-male triplets were delivered 5 days earlier than all-female triplets:

Type of Litter	*Mean Gestation Period (days)*		*Difference*
	M	*F*	*(days)*
Singleton	280·3	280·8	0·5
Mixed-sex twins	262·2	262·2	..
Same-sex twins	261·8	260·9	0·9
MFF triplets	245·6	245·6	..
MMF triplets	246·6	246·6	..
Same-sex triplets	244·6	249·6	5·0

It is the all-female triplets who are at variance, mixed triplets having a gestation-time comparable with that for all-male triplets. This longer gestation period may account for the small difference in birth-weight between all-male and all-female triplets.

All mixed sibships are dizygous, but some same-sex sibships will be monozygous. For both sexes, the infant from the mixed sibship was larger than the infant from the same-sex sibship. The difference was, however, much larger (0·43 lb.) for males than for females (0·15 lb.). Female twins from a mixed sibship were larger even than males from an all-male sibship. This suggests that not only does the presence of a Y chromosome enhance the growth-rate of the whole conceptus, but that it is either more potent, antigenically, than autosomal variety, or that its presence is necessary for the greater autosomal variety of dizygosity to be effective in enhancing fetal growth-rate.

Triplets may result from multiple ovulation or from polyembryony.[10] In polyembryony, the infants will inevitably be all of the same sex, although some same-sex triplets, like all mixed-sex triplets, are likely to be combinations of monozygotic and dizygotic twinning, and some will be polyzygotic. When, in a mixed sibship, one infant is of different sex from the others, this infant must be the result of multiple ovulation. Where two members of one sex are in a mixed sibship some will be the result of polyembryony.

The mean birth-weights of triplets, arrayed in descending order of magnitude, are:

Type of Litter	*Propositus*	*Mean Birth-weight (lb.)*	*Mean Gestation (days)*
(*a*) MFF	M	4·22	245·6
(*b*) FMM	F	4·19	246·6
(*c*) MMF	M	4·01	246 6
(*d*) FFM	F	3·94	245·6
(*e*) MMM	M	3·92	244·6
(*f*) FFF	F	3·88	249·6

Perhaps the most striking finding is the strict alternation of the sexes with decreasing birth-weight. The single females (*b*), who cannot be monozygous, were only 0·03 lb. lighter than the heaviest males (*a*). They were 0·18 lb. heavier than the next males (*c*) some of whom will be monozygous. The differences between the largest (*a*) and the smallest (*e*) males (0·30 lb.), and the largest (*b*) and smallest (*f*) females (0·31 lb.) were almost the same. These findings support the hypothesis that monozygosity, where antigenic dissimilarity is least, reduces fetal growth-rate; and that the presence of the Y chromosome enhances fetal growth-rate, its effect operating on the whole conceptus.

REFERENCES

1. Ounsted, M., Ounsted, C. *Nature*, 1966, **212**, 995.
2. Ounsted, M. *in* Aspects of Praematurity and Dysmaturity (edited by J. H. P. Jonxis, H. K. A. Visser, J. A. Troelstra, and H. E. Stenfert Kroese), p. 167. Leiden, 1968.
3. Ounsted, M., Ounsted, C. *Nature*, 1968, **220**, 599.
4. Ounsted, M. *Devel. Med. Child Neurol.* 1965, **7**, 479.
5. Ounsted, M. *in* Perinatal Factors Affecting Human Development: Pan American Health Organization, Scientific Publication no. 185, p. 60. Washington, 1969.
6. Ounsted, M. *Devel. Med. Child Neurol.* 1969, **11**, 693.
7. James, D. A. *J. Reprod. Fertil.* 1967, **14**, 265.
8. Mittwoch, U. *Nature*, 1969, **221**, 446.
9. McKeown, T., Record, R. G. *J. Endocr.* 1952, **8**, 386.
10. Hamilton, W. J., Boyd, J. D., Mossman, H. W. Human Embryology; p. 151. Cambridge, 1962.

Methods and Devices

INTERMITTENT VENOUS BLOOD SAMPLING

ROBERT BROWN
University Department of Obstetrics and Gynæcology, Princess Mary Maternity Hospital, Newcastle upon Tyne NE2 3BD

THE estimation of serum hormone levels may require the taking of serial blood-samples either by multiple venepuncture or by some system of venous cannulation. Evidence points to intermittent venepuncture affecting hormone levels—for example, growth hormone.[1,2] Cannulation calls for only one venepuncture, which is much preferable to the patient. Any such system must (1) be acceptable to the patient over a period of time, and (2)

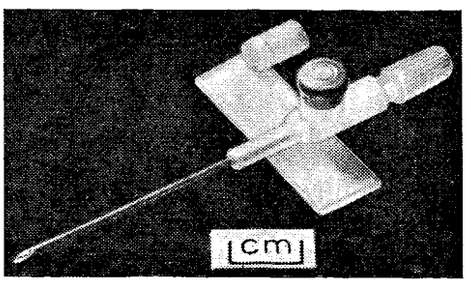

Fig. 1.—The cannula.