or chlorpromazine ("largactil"). Prednisolone (5 to 25 mg.) given before the ergotamine may be highly effective, relieving not only the nausea but the prostration of the attack if it is not altogether prevented.

2. Diuretics

The administration of these drugs is undoubtedly a most useful prophylactic measure in patients whose attacks seem to be related to water retention. Urea is the most useful diuretic, because it retains its efficacy and may be administered for life if necessary. It is best given in doses of 10–20 g. three times a day after food, dissolved in a little hot water flavoured with lime juice.

Urea (or carbamide, $\text{CO}(\text{NH}_2)_2$) is a natural product of the metabolism of protein and is completely without toxic effect. It often reduces the incidence of attacks of migraine and in occasional cases altogether prevents them. It is a much-neglected remedy.

3. Carbachol

Carbachol, *B.P.* syn. carbamylcholine chloride, $[\text{H}_2\text{N.CO.OCH}_2\text{CH}_2\text{N}+(\text{CH}_3)_3]\text{Cl}$ —("moryl"), is a cholinergic drug, and the rationale of giving it between the attacks is obscure. Yet its value is attested by several authors and by my own experience. It is given in a dosage of 2 mg. three times a day, and in some patients undoubtedly increases the intervals between attacks. It is contraindicated in the presence of cardiac failure and peptic ulcer.

4. Chlorpromazine

The main use of chlorpromazine (largactil) is to offset the toxic effects of ergotamine tartrate. 25 mg. should be given by mouth or by injection a quarter to half an hour beforehand. It can also be given as a suppository of 50 mg. Its use in acute attacks will be described later. "Stemetil" is a chemically related proprietary remedy which has not yet had an extended trial. As a prophylactic 10 mg. is given morning and evening.

(To be concluded)

What vaccinations do you need to travel abroad? The answer is to be found in the new publication of the World Health Organization (W.H.O.), *Vaccination Certificate Requirements for International Travel*, which lists the vaccinations imposed or recommended by some 180 countries and territories from Aden to Zanzibar. Land, sea, and air travel around the world comes under the International Sanitary Regulations promulgated by W.H.O. in 1952. Their aim is to ensure maximum security against the international spread of disease with minimum interference with world traffic. Under these regulations, certificates of vaccination against only three diseases—cholera, smallpox, and yellow fever—may be required. The necessity for one or more of these vaccinations varies according to the country from which the traveller comes and the itinerary he intends to take. The now famous yellow booklet of vaccinations, "The WHO International Certificate of Inoculation and Vaccination," has become as important for the traveller as his passport.

A FOETAL TISSUE BANK PROBLEMS AND PROSPECTS

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Two of the most fruitful branches of recent research have been concerned with tissue-transplantation immunity and with the effects of radiation on the body. Where these have overlapped there has developed the study of bone-marrow replacement, which, in this new age of radiation, is of obvious practical importance. It is hoped that by transplantation of fresh marrow cells after massive accidental irradiation lives may be saved and, in a similar way, that the scope of high-dosage radiotherapy could be extended.

At an early stage it became apparent that, for donor material, foetal cells might hold definite, even decisive, advantages over adult cells but that other factors might counterbalance these (see Table). Clearly more information was required.

Immunological Considerations

It can be stated as a principle that grafts of living cells exchanged between unrelated members of a species are destroyed after a short interval by an immune response of the host's lymphoreticular system—the homograft reaction. The main exceptions to this rule occur where either the graft is "insulated," as in the cornea, or where the host's immune system is ineffective

*Advantages and Disadvantages of Foetal and Adult
Haemopoietic Cells for Transplantation*

	Adult	Foetal
Quantity	Unlimited. (1) Donor (2) Cadaver	Limited
Quality:		
Activity	Normal adult proportions	Erythro > thrombo > leucopoietic
Stem cells	Low proportion	High proportion
Lymphoid cells ..	Small numbers in marrow	Precursors accompany haemopoietic cells in spleen and ? liver
Tolerance:		
Of graft by host ..	Normal homograft reaction unless modified by radiation, etc.	Homograft reaction possibly slightly delayed
Of host by graft ..	Seldom achieved. Usually "secondary disease"	Better than adult but sometimes "secondary disease"
Genotype availability	Wide range available either (1) fresh from donors or (2) stored from cadavers	Limited to stored tissue

owing to immaturity (in the foetus), disease (agammaglobulinaemia), or damage (irradiation, nitrogen mustards, etc.).

The dose of whole-body radiation needed to suppress the immune mechanism is very high, exceeding the dose which, without marrow replacement, would be fatal. With doses in this range, therefore, marrow replacement becomes simultaneously both possible and essential to survival. This is the basis of bone-marrow replacement therapy. After marrow-assisted recovery a rather complicated situation may develop. The following

events may occur: (1) The irradiated host tissues may slowly recover to form his own blood cells and at the same time the recovering lymphoreticular system may react against the grafted cells. Thus there is a slow return to the original ungrafted state, or, if this reaction is incomplete, there may be a mixture of circulating blood cells. (2) The grafted cells may remain and proliferate normally without reacting against the surviving cells of their new host, who thus becomes a stable chimera. (3) The grafted cells may react immunely against host cells which are foreign to them. This produces the condition of "secondary disease," characterized by wasting and diarrhoea, which is usually but not always fatal.

The factors determining the exact course of events are still somewhat obscure, but it would seem that the dose of radiation, the degree of "compatibility" (both ways) of host and graft, and the number of grafted lymphoid (immunologically competent) cells are, in combination, the determinants.

The virtue of foetal cells lies in their immunological immaturity, so that they are less capable of reacting against their host and causing the aftermath of secondary disease. They also have a second advantage, which remains theoretical and as yet unproved. After high doses of radiation, especially in the treatment of leukaemia, the fatal complication, despite all the measures that can be taken, is usually infection (Thomas, Lochte, and Ferrebee, 1959). This may be attributed in the main to lack of an effectual defensive system, and it is to be hoped that, in these circumstances, foetal lymphoid precursors, tolerant of their new hosts, may yet mature sufficiently to provide some antibacterial defences.

Collection of Human Foetuses

Unfortunately the supply of human foetal tissue is severely limited by stubborn practical considerations. Thus in the first three months of pregnancy the embryo is very small and is seldom to be had in an intact and sterile form. In the last three months, on the other hand, the infant, if not actually so, is held to be potentially viable, and parents' consent to a necropsy is necessary. Discretion in these circumstances calls for some delay, and thus it is seldom possible to obtain fresh tissues from late foetuses. Moreover, it is not yet known whether such foetuses retain their original virtue of adoptive tolerance.

There remains the middle period of pregnancy, from 12 to 28 weeks. A few pregnancies are terminated by hysterotomy at this time, mostly for renal, cardiac, or mental disease; spontaneous abortion is also uncommon. When it does occur a proportion of the foetuses are macerated, but in those that are not the tissues appear to be healthy. Occasional evidence of trauma is seen, and sometimes a foetus, though but recently dead, is found to be infected. With this exception, however, they can be taken as suitable donor material for tissue transplants.

The actual cause of these abortions is in most cases not discovered, and it is likely that a large number, perhaps the majority, are induced. In other cases gynaecological abnormalities may be present, and in some patients mid-pregnancy is the time that habitual abortion occurs. The possible role of blood-group incompatibility, especially in the ABO system, is discussed later.

Whatever the cause may be, foetal death does not precede delivery by very long. We have used, as a rapid

test of cell viability, Schrek's (1936) eosin-staining technique. Unstained cells in such preparations are considered viable, and with a suspension of hepatic cells the results are clear-cut. In comparing suspensions from hysterotomy specimens with those from "natural" abortions we have found no significant difference except when there has been delay in obtaining or dissecting the foetus. At room temperature six hours after delivery some decrease in viability is noticed, and at 12 hours most cells are dead. If kept at 4° C. cell death is very appreciably delayed.

It was soon evident that to obtain enough foetuses in a fresh condition an organization would be needed which must cover most of the Greater London area and be alert for 24 hours a day. That this has been achieved is due very largely to the help of the gynaecologists and nursing staff of the many hospitals concerned. On them has fallen the burden of remembering to inform us on the rare occasions when a suitable foetus is available.

Foetal Haemopoiesis

Blood cells in foetal life are derived from many sites of formation. From the accompanying diagram (Fig. 1) and Figs. 2-5 it will be seen that in the specimens available to us the liver is the main organ concerned. It should perhaps be emphasized also that, although there is some decline in the relative proportion of hepatic haemopoiesis after the 20th week, there is little absolute change until at least the 28th week. The spleen by

FIG. 1.—Foetal haemopoiesis. Diagram, reproduced by permission, from M. M. Wintrobe's *Clinical Hematology* (Fig. 1), 4th ed., 1956. Henry Kimpton, London.

FIG. 2.—Foetal liver at 12 weeks. The majority of cells are haemopoietic. ($\times 160$.)

contrast is both small and contains few haemopoietic cells, but by virtue of its lymphopoietic and some myelopoietic activity it may be of supplementary value. Bone marrow itself we have not attempted to use. The

FIG. 3.—Foetal liver at 18 weeks. A megakaryocyte can be seen on the right. ($\times 160$.)

FIG. 4.—Foetal liver at 28 weeks. Only islands of darkly staining haemopoietic cells remain ($\times 160$.)

FIG. 5.—Foetal spleen at 20 weeks. An incompletely formed follicle and a few islands of haemopoiesis are shown. ($\times 160$.)

total volume of such tissue is never large and is divided up among many bones from which extraction is difficult.

The foetal necropsy is performed with full sterile precautions, and, unless it has been delivered intact in its membranes, the whole skin surface is swabbed with tincture of iodine. The dissection presents no special technical difficulties, but to maintain an adequate exposure a pair of Cawthorne's mastoid retractors has been found useful.

The liver and spleen can be reduced to a cell suspension by pressing gently through a wire mesh of grid size $150\ \mu$. This removes all the stroma and breaks the tissue up into individual cells together with a few small clumps (Figs. 6 and 7). Such clumps can be eliminated by further meshing, but this practice has been discarded, as it damages the free cells. There is no evidence that the clumps are harmful. A sample of the suspension so obtained is cultured aerobically and anaerobically; the remainder is stored either at 4°C . for 12–24 hours or at -79°C . for longer periods.

Short-term culture of a sample of the cell suspension can be carried out at this stage using Lajtha's (1954) method, and this will give an indication of the activity of the cells—for example, their capacity to synthesize nucleic acid (D.N.A.). Unfortunately no method has yet been discovered of increasing the yield of useful cells by long-term culture.

FIG. 6.—Smear of foetal liver and spleen suspension. Myeloid, erythroid, and undifferentiated stem cells can be distinguished. ($\times 608$.)

FIG. 7.—Smear of foetal liver and spleen suspension to show megakaryocyte. ($\times 608$.)

Freezing and Thawing of Cells

Long-term storage of frozen cells is no longer a novelty. The work of Parkes and others, and, in particular, their discovery of the protective action of glycerol, has enabled many different types of cells to be preserved for months or years at the temperature of dry ice (-79°C.) or liquid nitrogen (-195°C.). But there is still ignorance of some of the principles involved.

Thus, in the case of spermatozoa, about which most is known, there is a wide variation in freezing optima from one species to another (Polge, 1957). For the rabbit 25–30% glycerol in buffered glucose is best, compared with 10% glycerol in glucose+egg yolk for the stallion; 10% glycerol is also the optimum for bull semen, but for man the concentration should be 0–5%. For the ram no good method has yet been discovered, and, although thawed raw spermatozoa may be actively motile, their fertility is always severely reduced. As well as these species variations there are undoubtedly different optima for different tissues within the same species and for cells of one tissue at different stages of maturation.

The standard preserving medium for bone marrow is 15% glycerol in serum, but there is no proof yet that this is the optimum for man as it probably is for the mouse. Ferrebee *et al.* (1957), for example, have shown that lower concentrations of glycerol are almost equally effective in mice and that no *in vitro* test is capable of predicting accurately the *in vivo* performance of frozen cells. Thus the capacity for D.N.A. synthesis in cultures of frozen and thawed mouse marrow is reduced by nearly 90%, although the cells are still capable of survival and proliferation *in vivo*. There are two plausible explanations for this observation. It may be that some time is needed for recovery after freezing and thawing which cannot be granted under experimental conditions *in vitro* as opposed to *in vivo*. Alternatively it must be remembered that ultimate survival depends on the very small proportion of primitive stem cells in the marrow, and perhaps it is these alone that continue to synthesize D.N.A. Their freezing optima may be significantly different from those of the semi-mature marrow cells. So in dealing with such a heterogeneous population of cells, as is found in foetal liver, some uncertainty remains. Using the technique of autoradiography, we can show that D.N.A. synthesis continues after freezing and thawing in 15% glycerol-serum, but what proportion of the all-important stem cells have survived, and whether we have given them the very best chance of survival, is still unknown.

Storage.—Mere storage of frozen tissue presents few problems. Alternative methods employ either liquid nitrogen, dry ice crushed or dissolved in alcohol, or a mechanical refrigeration plant. A combination of these methods is possible. The choice depends on circumstances, chiefly the availability of a supply of dry ice or liquid nitrogen. In London at a cost of less than £100 per annum for dry ice it should be possible to store 5 litres of tissue-suspension or about 3×10^{12} cells.

Administration.—Suspensions of haemopoietic cells can be given intravenously without hazard provided the injection is made slowly. Frozen cells should always be thawed as quickly as possible and injected without delay. By virtue of some extraordinary homing instinct it has been shown experimentally that cells so

administered find their way to the appropriate site in the body, where they settle and multiply.

Some Properties of Foetal Cells

Foetal Blood Groups.—Contrary to general belief, most antigens of foetal blood cells are well developed at an early stage and probably, indeed, from their very origin. The only important exception to this seems to be the ABO system, where there is some qualitative defect; the character A_1 , for example, is never manifest before birth. There is thus no difficulty in genotyping foetal erythrocytes, and our routine practice is to determine the ABO, D, C, E, $\bar{c}$, $\bar{e}$, M, N, S, Fy^a , and K groups. It would not be surprising if an excess of certain blood groups was found, since incompatibility of mother and foetus might be the cause of some of these abortions. ABO antigen differences are under particular suspicion of this sort, but hitherto our figures have revealed, if anything, an excess of group O foetuses—that is, the opposite of what one might expect.

Transplantation of Other Foetal Tissues.—There is evidence that certain foetal cells, when transplanted, can overcome the normal homograft barrier. Endocrine glands have received most attention, and many believe that parathyroids, at least, can survive and function in their new host (Jordan, Foster, and Gyorkey, 1958). If this was proved to be so, we could perhaps look forward to the prospect of a new radical form of endocrine therapy equivalent to a sort of “spare parts” service.

Research Opportunities

Many forms of research in human biology are dependent on a supply of foetal tissues. Tissue culture, for example, succeeds most often with early rapidly growing cells, and on such cultures viruses may in turn proliferate, thereby yielding results of great practical importance, including the material for making vaccines.

The growth and development of the human foetus itself is still only a half-charted territory; Willis (1958) pointed out some of the deficiencies. In this field we have been able to assist research workers in exploring the development of the lungs, the nervous system, alimentary tract, breast, and other tissues, and in measuring the accumulation of radioactive substances in the foetal bones. Others have studied foetal haemoglobins, plasma proteins, and the emergence of various enzyme systems.

If so little is known of the normal human foetus, what of the abnormal? Only isolated descriptions are available of the early stages of many abnormalities. We may hope in time by careful search to discover specimens of deformities—for example, hare-lip—at a stage nearer to their origin, whence there could be derived information of use in treatment or even prevention.

Firstly, of course, we are indebted to all who have helped to provide us with foetuses. This work has latterly been supported by a grant from the Medical Research Council, and we must acknowledge the recent assistance of Dr. G. Barraclough. We also thank Mr. K. G. Moreman for the photomicrographs.

REFERENCES

- Ferrebee, J. W., Billen, D., Urso, I. M., Wan Ching Lu, Thomas, E. D., and Congdon, C. C. (1957). *Blood*, **12**, 1096.
- Jordan, G. L., Foster, R. P., and Gyorkey, F. (1958). *Plast. reconstr. Surg., Transplant. Bull.*, **22**, 392.
- Lajtha, L. G. (1952). *J. clin. Path.*, **5**, 67.
- Polge, C. (1957). *Proc. Roy. Soc., B*, **147**, 498.
- Schrek, R. (1936). *Amer. J. Cancer*, **28**, 389.
- Thomas, E. D., Lochte, H. L., jun., and Ferrebee, J. W. (1959). *Blood*, **14**, 1.
- Willis, R. A. (1958). *The Borderland of Embryology and Pathology*. Butterworth, London.