Fetal tissue/organ transplant in HLA-randomized adult vascular subcutaneous axillary folds: preliminary report of 14 patients

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Summary

Background: In the year 1902, the first successful experimental organ transplant, i.e., an autotransplant of a dog's kidney from its normal position to the vessels of the neck, which resulted in some urine flow, was performed in the Vienna Physiology Institute under the direction of Hofrath Exner by Dr. Emerich Ullman (1861-1937). Since then, the art of transplant surgery has come a long way in establishing itself as an important discipline with the support disciplines of immunology, molecular biology, etc., for the restoration of a failing organ. Today there is a major discrepancy in the demand and supply of organ grafts. The aim of the present study is to see whether fetal organ and tissue, with its intrinsic advantages of hypo-antigenicity, can survive in a HLA and sex-randomized host in a surgically prepared vascular subcutaneous axillary fold, without any immunosuppressive support. We have earlier reported two cases of fetal thymic transplant, collected from consenting mothers undergoing hysterotomy and ligation.

Materials and methods: Fourteen cases were recruited for the present study after thorough informed consent and approval by the Ethical Committee of the Project. Of these, five patients were suffering from advanced cancer, three from diabetic gangrene, three from ischaemic heart disease and three from rheumatoid arthritis, liver abscess and disc prolapse. The ages of the patients varied from 39 to 82 years. Six fetal thymuses, three fetal liver tissues, three fetal cardiac tissues, one fetal pancreas and one fetal lung tissue were transplanted. All the fetuses were dissected and the selected tissues/organs were transplanted within one to three minutes after collecting them from the consenting mothers undergoing hysterotomy and ligation. The fetal tissue graft was placed in a surgically prepared subcutaneous vascular axillary fold, 2x1 cm, under local anaesthesia in the consenting adult recipient. Sequential Hb, Tc, Dc, ESR were done to see the impact of the transplant on the host system. After one month, the transplanted fetal tissue was taken out by an elliptical incision and the tissue was processed for histological staining.

Results and analysis: All the 14 patients tolerated the transplant procedure well. There was no fever, intractable pain or any other specific serious side-effect justifying removal of the transplant earlier. There was no discharge from the incision site and the healing and scar were by and large normal. There was no unusual leucocytosis or lymphocytosis. The serial histological study did not suggest features of transplant rejection.

Discussion and conclusion: Pregnancy and neoplasm are two outstanding examples of natural tolerance to homograft. In both cases, blocking antibody has an important role in the phenomenon of immunotolerance. From our experiments mentioned above transplantation and our earlier reported studies, we believe that the hypo-antigenic fetal tissue has distinct advantages over adult tissue for transplant purposes.

Key words: Transplantation cardiac; Lung; Liver; Thymus; Fetal tissue growth; HLA-randomized adult axilla.

Introduction

In the year 1977, in the course of certain experimentation on the acquisition of immunocompetence of the human fetus, we were surprised to note that intraamniotic instillation of an antigen, that is, tetanus toxoid, could cause abortion [1]. That the effect is not specific to tetanus toxoid was confirmed when abortion was induced as a result of the instillation of other antigens like double antigen, maternal buffy-coated leucocyte of 10 ml blood, etc. [2]. While antigen stimulation of the developing fetal humoral immune system can provoke abortion, stimulation of the developing fetal cellular immune system by intraamniotic instillation of BCG can cause dissolution and autoabsorption of the human fetus [3-6]. In the case of stimulation by instillation of intraamniotic 2 cc tetanus toxoid, we observed that the induction-abortion interval varied widely, i.e., 92.8% of the mothers in our study aborted within one month, which fell to 86.2% within 21 days, 72.4% within two weeks and 52.8% within one week. All consenting mothers were admitted for hysterotomy and ligation and the experimental protocol was passed through the ethical committee of the institute. One major problem which remains to be solved with this intraamniotic tetanus toxoid experimentation is the wide fluctuation in the induction-abortion interval.

Why the allogenic fetus is not rejected by the mother remains a mystery. The possible reasons [7] could be summarized as: (a) a mild state of immunosuppression in pregnancy, (b) poor allo-antigenic expression of the trophoblast, (c) the trophoblast contains some strong locally acting cytokines and growth factors which suppress the immune response mechanism, (d) the placenta is resistant to attack by the maternal antibody or cell-mediated damage due to the presence of a non-specific blocking antibody.

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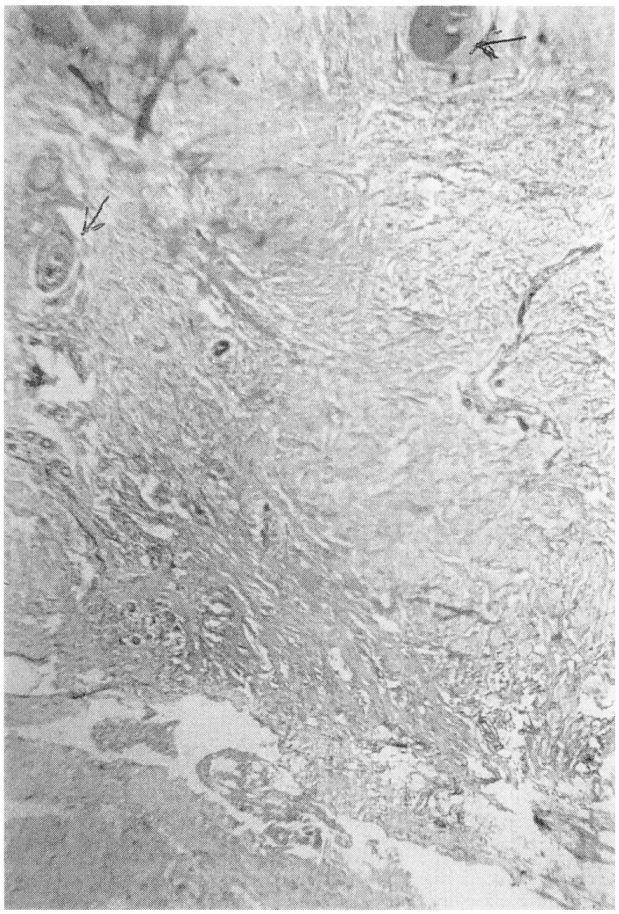


Figure 1. — A photomicrograph showing a 20-week fetal myocardial tissue implanted under the skin. Axillary hair follicles can be identified. The mesenchymal cells and proliferating myoblast of the fetal heart is not disturbed by the host's inflammatory or immunological rejection phenomenon, i.e., thrombosis, mononuclear invasion, endarterites or vascular disruption. This photograph is taken under low power microscope with HE (haematoxylin and eosin) stain.

On the basis of our experience over the past two decades on the reactions of the fetal immune system to an intraamniotic antigenic assault, we can say that the pre-immune (within 15 weeks) and hypo-immune (after 15 weeks) developing fetal immune systems provide certain intrinsic advantages of the fetal tissue as a graft for transplants. We have earlier reported on the safety of (174 units) umbilical cord whole blood transplantation in 62 patients [8], which we have been following-up for the last two years. What is worthy of note is the fact that after following the standard blood transfusion protocol, there was no immunological or non-immunological reaction in any patient. In a subsequent set of experiments on orthotopic fetal thymus transplant in the axilla of cancer patients, we noted with surprise that there was growth, differentiation and even interaction with the host immune status [9].

Materials and Methods

Whether the privileges of the hypoantigenicity of the human fetal tissue can be used for universal cell or tissue transplant in case of need for the restoration of the functions of a diseased organ, is the rationale behind the present transplantation protocol. In the current set of experimentations on a simple orthotopic transplantation site at the axilla under local one percent xylocaine infiltration anaesthesia, a little fetal tissue/organ is placed in a vascular subcutaneous 2x1 cm dissected space. The experiments were preceded by written informed consent by the patient/guardian and after the completion of the necessary formalities of the hospital ethical committee. A second consent was taken from the donor mothers admitted for hysterotomy and ligation who contributed the fetal tissues. Hysterotomy was conducted in the OR under general anaesthesia following standard preoperative, operative and postoperative text book protocol and the fetus with the intact sac was taken out judiciously by the first group of surgeons. Care was taken not to rupture the amniotic cavity. In the same OR, simultaneously at an adjacent table, a second group of surgeons prepared the recipient's axilla, after infiltrating subcutaneously 1% xylocaine, and after repeatedly washing with rectified alcohol and betadine. All other standard protocol of antiseptic/aseptic dressing and draping were followed meticulously. Then, the second group of surgeons dissected a 2 x 1 cm space in the subcutaneous tissue of the prepared axilla and controlled the bleeding adequately. The second group of surgeons then waited for the first group of surgeons to hand over the fetus with the intact amniotic sac and placenta. Within a minute, the second group of surgeons then dissected the fetus and took out the tissue/organ from the thymus/liver/lung/pancreas/cartilage/heart, as per decisions of transplantation. After the placement of the fetal tissue graft in the adult axilla, the skin was closed with interrupted (900) atraumatic vicryl sutures and a small dressing was applied to cover the incision site. After a one-month observation period, the transplanted fetal tissue was taken out by an elliptical incision, 2 x 1 cm, under local anaesthesia following the standard antiseptic/aseptic protocol of surgery in the OR. A periodic assessment of Hb, Tc, Dc, ESR was done at the pre- and post-transplant phases, as reported earlier [10].

Results and analysis

The following table depicts our experience with pre-immune and hypo-immune fetal tissue transplant at the axillary site of HLA and sex-randomized adults with neoplastic and non-neoplastic disease backgrounds.

These transplants were conducted on our patients in Bijoygarh State Hospital. As per the ethical committee's strict protocol, in no case was the primary care and treatment of the patient jeopardized as a result of the experiment protocol. We strictly followed duty-based ethics and the traditional concept of doctor-patient relationship relying on the principle of (a) do no harm, (b) try to do good, (c) respect for autonomy, (d) justice, also keeping in mind the concept of utilitarianism in a broader view.

In all these 14 cases, though the axillary transplant site was healthy, we removed the transplanted tissue after one month with an elliptical incision under local anaesthesia and subsequently stained the removed tissue with haematoxylin and eosin (HE), to examine the impact of transplantation of the fetal tissue (sex-randomized) in the adult host and *vice versa.*

Patient age	Suffering from	Type of fetal grafted tissue at adult axilla	Serial study of Hb/Tc/Dc/ESR on 1st/3rd/5th/7th/14th/21st/28th days post-transplant	Other supportive treatments	Comments on the local axillary transplant site
1. Mrs. L. D. [45]	Gall Bladder Cancer, Stage IV	14-week fetal liver tissue	Very little change from pre-transplant level	Cephalexin and NS-AID*	Healthy
2. Mrs. M. S. [46]	Infiltrating duct carcinoma, Stage III	14-week fetal total thymus	No major change (more than 20%) from pre-transplant level	Cephalexin and NS-AID*	Healthy
3. Mrs. S. M. [65]	Adeno-carcinoma of the ovary, Stage IV	Total thymus of 20-week fetus	No major shift	–do–	Healthy
4. Mr. S. D. [40]	Adeno-carcinoma of the lung, Stage IV	Lung tissue from a 20-week fetus	–do–	–do–	Healthy
5. Mrs. P. M. [39]	Infiltrating duct carcinoma, Stage III	Liver tissue from a 10-week fetal liver	–do–	–do–	Healthy
6. Mr. K. K. C. [82]	Diabetic gangrene with poor GC	Total pancreas and spleen from a 20-week fetus	–do–	Act-rapid human insulin, pentoxyphylline drip, vitamins, cephalexine and other supports	Healthy
7. Mr. T. H. [79]	COPD with ischaemic heart disease	Cardiac tissue from a 20-week fetus	–do–	Salmeterol inhalation with nitrates and calcium blocker, etc.	Healthy
8. Mr. G. N. [54]	Disc prolapse	Thymus of a 20-week fetus	–do–	Traction, vitamins, NS-AID*	Healthy
9. Mr. M. S. [38]	Amoebic liver abscess	14-week fetal liver tissue	–do–	Anti-amoebic drug (ornidazole) and tetracycline, etc.	Healthy
10. Mr. T. D. [49]	Ischaemic heart disease with old infarction	10-week fetal cardiac tissue	–do–	Nitrates, beta-blockers, aspirin	Healthy
11. Mr. A. S. [45]	Diabetic gangrene	Fetal thymus, 20 weeks	–do–	Act-rapid human insulin, antibiotics, pentoxyphylline drip	Healthy
12. Mr. R. T. [49]	Diabetic gangrene	Fetal thymus, 20 weeks	–do–	–do–	Healthy
13. Mrs. S. T. [49]	Ischaemic heart disease	Fetal cardiac tissue, 16 weeks	–do–	Nitrates and beta-blockers, aspirin	Healthy
14. Mrs. K. B. [55]	Rheumatoid arthritis with deformity	14-week fetal thymus	–do–	NS-AID* and chloroquine with physiotherapy	Healthy

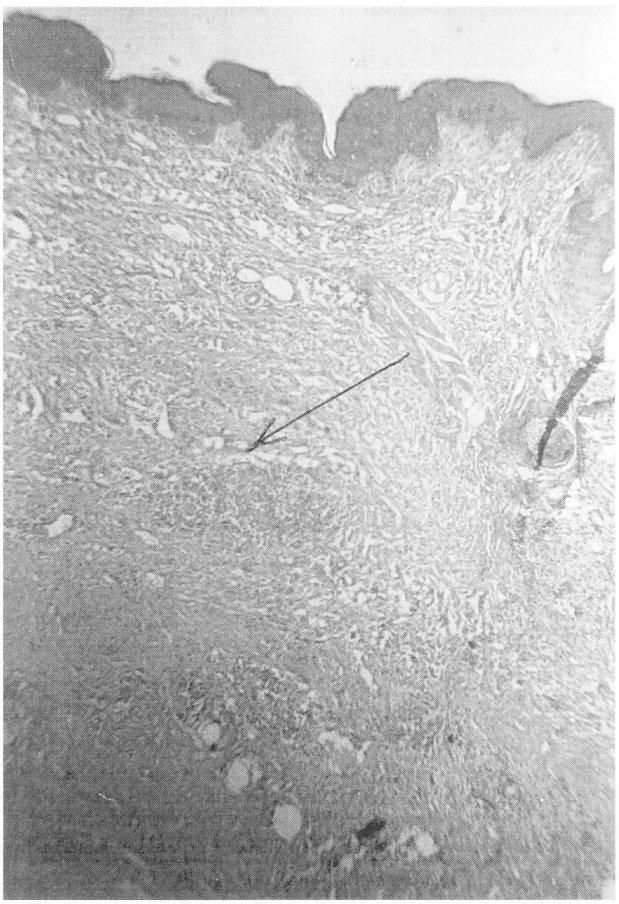
• = Non-steroidal anti-inflammatory drug.

We know that the cornea and cartilage are readily accepted as grafts. We also know that the deciduas of the uterus provide a certain degree of environmental privilege for the growth of the allogenic fetus, but the axilla has never been cited in medical literature as a privileged site. The serial studies of the histological specimens did not reveal features of acute rejection and there was no sign of ischaemic damage of the graft-like thrombosis, mononuclear invasion, endothelial disruption or endarteritites, etc. On the contrary, there was clinical evidence of increased vascularity of the graft and its surroundings. The question is, why is this so.

Fetal growth is dependent on a unique symbiotic environment where the mother provides all the necessary factors and environment for growth, proliferation and differentiation. The fetal micro-environment is distinctly different from the adult neuro-endocrine and metabolic micro-environment [11]. Hence, it is possible that the fetal transplanted graft tissue adjusts its own micro-environment to an altered metabolic environment, i.e., in the adult, using the advantage of its hypo-immune or pre-immune status in order to survive, grow, proliferate and differentiate further. Another interesting point to note is the perfect healing of the transplant site surgical wound. Why and how transplanted fetal tissue in sex and HLA-randomized adults escapes immunological recognition and becomes a human homologous chimera still remains a mystery.

In addition, a study of the histological material did not suggest any foreign body giant cell or other similar response. Sequential haemoglobin, Tc, Dc, ESR studies on the recipients of transplants did not show any major variation when comparing the pre- and post-transplant results. There was no gross variation in the pre- and post-transplant leucocyte or lymphocyte counts.

Fig. 2



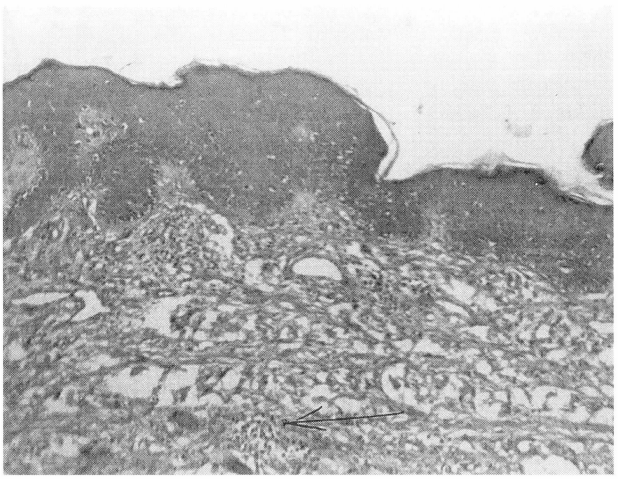


Fig. 3

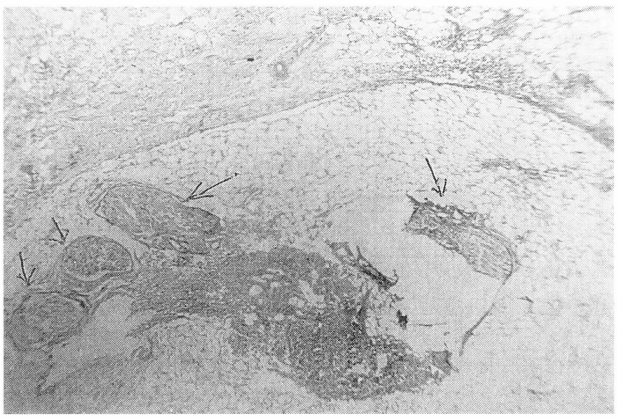


Fig. 4

Figures 2, 3, 4. — HE-stained microphotographs showing 14-week (pre-immune) thymuses - Scan power: Photomicrograph No. 2, and Low power: Photomicrograph No. 3, and another thymus (20 weeks) under Scan power - all of which have been implanted under the axillary skin. The thymus develops from epithelial cells of the endoderm of the third pair of pharyngeal pouches and from the mesenchyme into which the epithelial cells grow. Some of the epithelial cells, at 15-16 weeks, arrange to form a group of cells known as thymic (Hassall's) corpuscles. There are no features of mononuclear invasion, thrombosis, or endarterites justifying rejection of the fetal transplanted thymuses from the adult axilla.

Discussion

The basis for transplant immunology was laid by Sir Peter Medwar in a series of experiments from 1944 onwards using mainly skin transplant techniques. He formulated the basic tenets of transplant immunology like donor specificity of graft rejection, cell-mediated first-set response and antibody-mediated second-set response [12]. The rapid development of clinical organ transplantation over the past 40 years has often out-stripped our knowledge of the mechanisms involved. The immune system's need to differentiate between self and non-self (eg., bacteria) with distorted or perverted self (tumour or virally infected self), allows the organism to survive in this hostile environment. The molecular mechanism of self and non-self recognition resides in specialized cell-surface molecules known as major histocompatibility antigen (MHC). The first MHC was recognized in 1958 with a serological identification attempted in the line of ABO blood group system. Hence, it was promptly renamed as "Human Leucocytic Antigen" (HLA) [13]. This is now known as HLA-A2 and can be traced in the short arm of chromosome-6. The MHC has two sub-sets, i.e., the Class-I region and the Class-II region [14]. The Class-I region is divisible into HLA-A, B, C, allelic series (as well as E, F, G, and H) [15], of which A and B are thought to be important for transplantation purposes. Class-II is divided into DR, DP, and DQ loci [16], though only DR is used for matching purposes. Between Class-I and Class-II, there is a region known as Class-III which contains more than 70 genes to control the complement system (C2, C4, tumour necrotic factor alpha, tumour necrotic factor beta, etc.) [17].

While the molecular mechanisms discussed above in brief can explain the mechanism of transplant rejection of an adult organ, the phenomenon is far from clear so far as the hypo-immune or pre-immune fetal tissue transplant survival mechanism in adults is concerned, at least over a one-month period of observation in the present set of experiments in HLA-randomized hosts without any concommitent immunosuppressive drug support to delay rejection. From the HE-stained histological photomicro-

Fig. 5

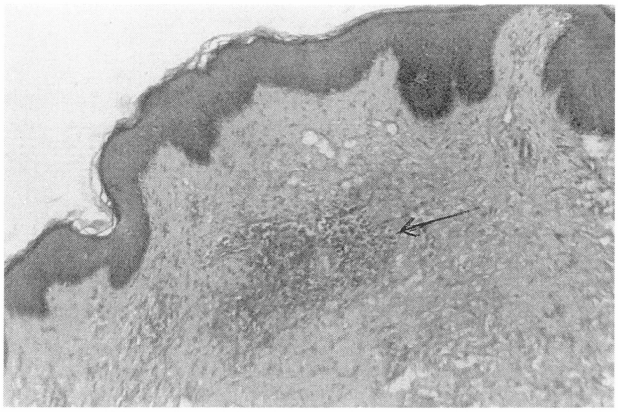
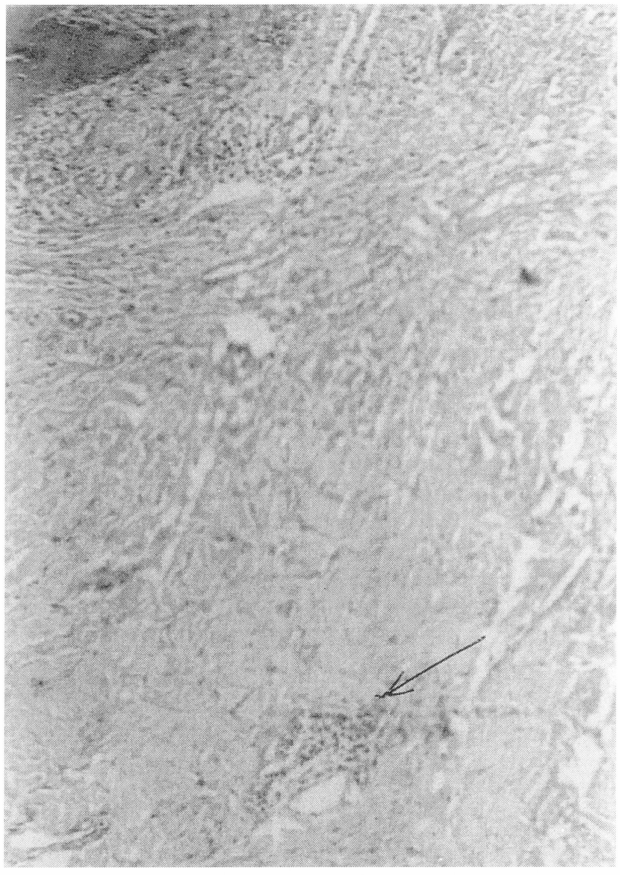


Fig. 6

Fig. 7



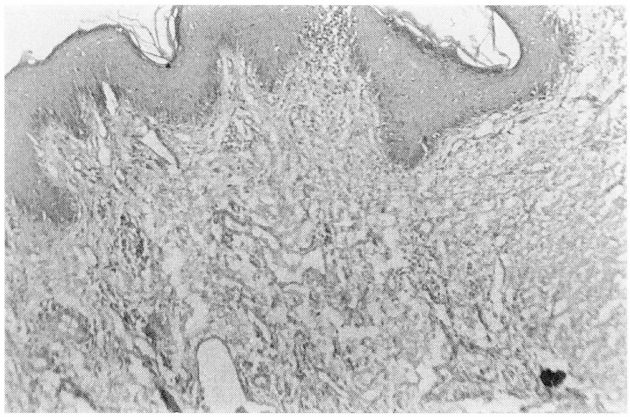
Figures 5, 6, 7. — HE-stained microphotographs showing 10-week and 14-week fetal liver tissue under Low power (Microphotograph 5, 10-week fetus), and Scan power (Microphotograph 6, 14 weeks), and Low power (Microphotograph 7, 14 weeks). Here, too, the photographs of the fetal liver tissue in the adult host do not suggest any features of rejection. In this connection, it is worth mentioning that haematopoisis of the fetal liver starts from 7 to 10 weeks, and bile formation starts from the tenth week. Bile itself can provoke an inflammatory response, which is not visible in these pictures.

graphs of the thymic tissue, liver tissue, cardiac tissue, pancreas and lung tissue of fetuses (within 20 weeks) transplanted into adult axillary fold incision sites, there did not appear to be any feature of rejection within the one-month observation period.

What, then, is the importance of this study? What are the futuristic implications of the research? According to one report from the United States, there is increasing disparity between the demand and supply of organs needed for patients awaiting organ transplantation. While the number of patients awaiting transplants increases at approximately 15% per year, the number of cadaver organ donors increases at only 1-3% per year. Actually, the number of cadaver organ donors in 1999 increased less than one percent over that in 1998 [18]. Whether fetal tissue/organ/cellular transplant, with its intrinsic advantages of hypo-antigenicity and hypo-immune status, can fill up the existing gap in the demand and supply of organs required for transplants, is a matter under intense scientific study. The preliminary report of our study indicates a positive direction in this field, in our long quest to understand the dimensions of the acquisition of immunocompetence by the human fetus and its manipulation in health and disease.

Another interesting development in the past 50 years is the attempted use of bio-ionic devices and solid organ transplants as a replacement for a diseased or failing tissue or organ, based on the principle of cell transplant

Fig. 8



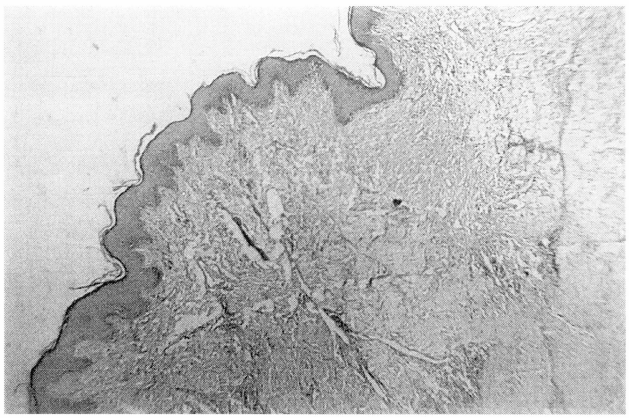
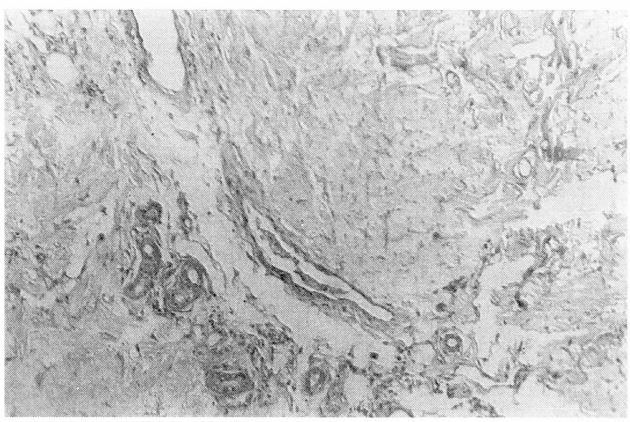


Fig. 9

Figures 8, 9. — HE-stained microphotographs showing 20-week fetal lung tissue transplanted under the adult axilla and viewed under Low power (Microphotograph 8) and Scan power (Microphotograph 9). At 20 weeks, the fetal lung development passes through the canalicular period (16 to 24 weeks). Once again, the features of rejection of the fetal tissue transplant are not observed.

Fig. 10



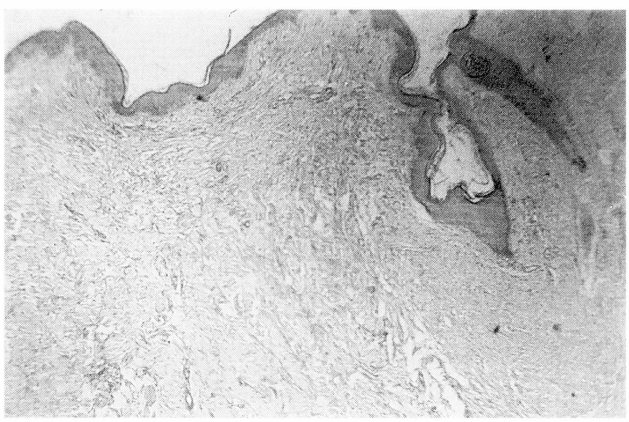
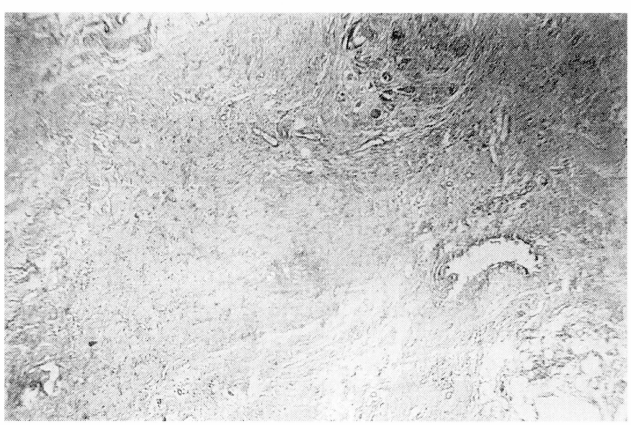


Fig. 11

Fig. 12



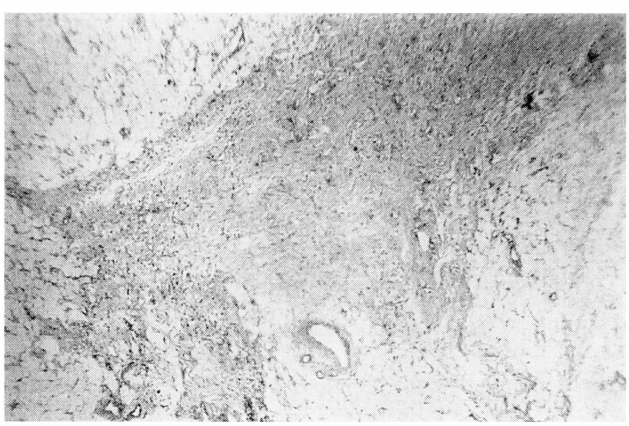


Fig. 13

Figures 10, 11, 12, 13. — HE-stained microphotographs showing the fate of a 20-week total fetal pancreas transplant in a HLA-randomized host suffering from diabetic gangrene. Microphotograph no. 10 is a Low power depiction of the pancreas. Microphotographs nos. 11 to 13 show proximal (no. 11), middle (no. 12), and distal (no. 13) parts of the transplanted pancreas. Classical features of immunological rejection are also not present in these pictures.

and regeneration in vivo with bio-artificial hypo-antigenic synthetic support. Cell transplants have been used successfully in Parkinsonism, to restore articular cartilage, etc. With the support and wisdom of the bio-engineering department, there have been attempts to control uncontrolled diabetes by implanting a bio-artificial pancreas consisting of islet cells micro-encapsulated in alginate. There is a fascinating review by Stocum, on the topic, "Regeneration Biology and Engineering: Strategies for Tissue Restoration" [19]. In fine, if the entire medical community unites on this very complex issue of fetal tissue-adult tissue transplant interaction in health and disease, there may be a major breakthrough in this hitherto vastly unknown and fascinating field.

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References

[1] Bhattacharya N., Chaudhury N., Banerjee S., Mukherjee K. L.: "Intraamniotic Tetanus Toxoid as a Safe Abortifaecient". *Indian J. Med. Res.*, 1979, *70*, 435.

[2] Bhattacharya N.: "Letter to the Editor". *Clin. Exp. Obstet. Gynecol.*, 1996, *23*(4), 272.

[3] Bhattacharya N.: "Intraamniotic instillation of tetanus toxoid: A safe, cheap, effective abortifaecient in the light of our experiences with different intraamniotic instillation of antigens for alteration of pregnancy immunotolerance – A study from 1978-1996". In: R. L. Tambiraja and N. K. Ho (eds.) "Relevance and Excellence in Perinatal Care", Proceedings of the Ninth Congress of the Federation of the Asia and Oceania Perinatal Societies, Singapore, 10-14 November 1996, Monduzzi Editore, Bologna, Italy, 193.

[4] Bhattacharya N.: "Dissolution of the fetus: A new experience with intraamniotic BCG instillation". *Ibid*, 201.

[5] Bhattacharya N.: "Study of the aborted fetuses after intraamniotic instillation of tetanus toxoid". *Ibid*, 187.

[6] Bhattacharya N.: "A study on the intraamniotic instillation of tetanus toxoid on a growing human fetus". Dissertation submitted to the Calcutta University (India), for the degree of Doctor of Science (D. Sc.), Faculty of Medicine, 2001.

[7] Halloran P. F.: "Tissue transplantation". In: J. B. Walter, I. C. Talbolt (eds.), "Walter and Israel General Pathology". 7th edition, Churchill Livingstone, New York, 1996, 281.

[8] Bhattacharya N., Mukherjee K., Chettri M. K., Banerjee T., Mani U., Bhattacharya S.: "A study report of 174 units of placental umbilical cord whole blood transfusion in 62 patients as a rich source of fetal hemoglobin supply in different indications of blood transfusion". *Clin. Exp. Obstet. Gynecol.*, 2001, *28*(1), 47.

[9] Bhattacharya N., Mukherjee K. L., Chettri M. K., Banerjee T., Bhattacharya S., Ghosh A. B., Bhattacharya M.: "A unique experience with human pre-immune (12 weeks) and hypoimmune (16 weeks) fetal thymus transplant in a vascular subcutaneous axillary fold in patients with advanced cancer - A report of two cases". *Eur. J. Gynecol. Oncol.*, June-July, 2001 (in press).

[10] Bhattacharya N. *et al.*: Ibid.

[11] Miller R. K.: "Fetal drug therapy: principles and issues". In: M. R. Pitkin and J. R. Scott (eds.), *Clinics in Obstetrics and Gynecology*, 1991, *34*(2), 241.

[12] Medwar P. B.: "The behaviour and fate of skin autografts and skin homografts in rabbits". *J. Anat.*, 1944, *78*, 176.

[13] Dausset J.: "Iso-leuco-anticorps (iso-leuko-antibodies)". *Acta Haematologica*, 1958, *20*, 156.

[14] Trowsdale J., Ragoussis J., Campbell R. D.: "Map of human MHC". *Immunol. Today*, 1991, *12*, 433.

[15] Koller B. H., Geraghty D. E., Demars R., Duvick L., Rich S. S., Orr H. T.: "Chromosomal organization of the human major histocompatibility class I gene family". *J. Exper. Med.*, 1989, *169*, 469.

[16] Dunham J., Sargent C. A., Dawkins R. L., Campbell R. D.: "An analysis of variations in the long range genomic organization of the human major histocompatibility class II region by pulse field gel electrophoresis". *Genomics*, 1989, *5*, 787.

[17] Trowsdale *et al.*: no. 14.

[18] Howard R. J.: "Transplantation". Comments in E. M. Copeland III, K. I. Bland, E. A. Ditch, T. J. Eberlin, R. J. Howard, E. A. Luce, J. M. Seeger, W. W. Souba, D. J. Sugarbaker (eds.), "Yearbook Surgery", 2000 (Mosby Inc., 2000), 93.

[19] Stocun D. L.: "Regenerative biology and engineering: Strategies for tissue restoration". Comment in "Wound Repair and Regeneration", 1998 *6*(4), 273.

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