A unique experience with human pre-immune (12 weeks) and hypo-immune (16 weeks) fetal thymus transplant in a vascular subcutaneous axillary fold in patients with advanced cancer: A report of two cases

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Summary

Background: The successful development of fetal cell/tissue transplantation in adults has resulted in the possibility of eventual therapeutic solutions with a variety of intractable diseases. Umbilical cord whole blood transplantation appears to be safe in the adult system. In severe forms of DiGeorge Syndrome, cultured thymus transplant can help in the reconstitution of the immune condition of the host. Successful fetal tissue transplant in adults has raised the hope of future effective gene transplant and its manipulation prospects to combat many diseases including hemopathies, inborn errors of metabolism, immunodeficiencies and even cancer and AIDS.

Materials and Method: Two cases of advanced cancer were treated with fetal (pre-immune 12 weeks and hypo-immune 16 weeks) thymus transplants in subcutaneous vascular axillary folds, which were removed after one month. Thymuses were collected from consenting mothers undergoing hysterotomy and ligation.

Results and Analysis: Patient 1 was suffering from non-Hodgkins lymphoma (Ann Arbor Stage IV) and was receiving cyclophosphomide, doxorubicin, vincristine and prednisolone after a course of radiotherapy; she developed leucopenia (2,400/cmm), which improved after receiving a 16-week human fetal thymic graft. The leucopenia was eventually over-corrected and the leucocyte count reached 44,000/cmm within a month, which was reversed after the thymus was taken out. Histology of the excised thymic graft showed growth and proliferation without any graft vs. host (GVH) reaction. Patient 2 was suffering from breast duct carcinoma (T_4, N_2, M_0) with estrogen, progesterone, and epidermal growth factor negative status, and was treated with modified radical mastectomy and axillary clearance followed by chemotherapy with cyclophosphomide, methotrexate and 5-fluorouracil for six cycles. She also received a 12-week-old human fetal thymus at the contra-lateral axilla which was removed after one month. In this case the peripheral leucocyte count did not show appreciable variation as in the first case. However, histology of the excised thymic graft showed growth and proliferation with an appearance of Hassel's corpuscles.

Conclusion: Pre-immune and hypo-immune human fetal thymic transplant is not rejected in patients suffering from advanced cancer within one month (observation period). Thymic lymphocyte shedding in the correction of leucopenia in the background of non-Hodgkin's lymphoma may have many therapeutic implications.

Key words: Human pre-immune (12 weeks) and hypo-immune (16 weeks) fetal thymus transplant can grow and proliferate in advanced cancer patients.

Introduction

During intrauterine growth, the human fetus passes through the pre-immune phase (before 15 weeks) and subsequently, through the hypo-immune phase of growth and maturation. The expression of hypo-antigenicity of the growing fetus in utero provides an excellent opportunity for fetal tissue/organ transplantation. Recent reports suggest that umbilical cord whole blood transplantation appears to be safe in cases of adults in need of whole blood transfusions [1]. In severe forms of DiGeorge syndrome, thymus or cultured thymic cells can help in the re-constitution of the immune condition of the host [2-10].

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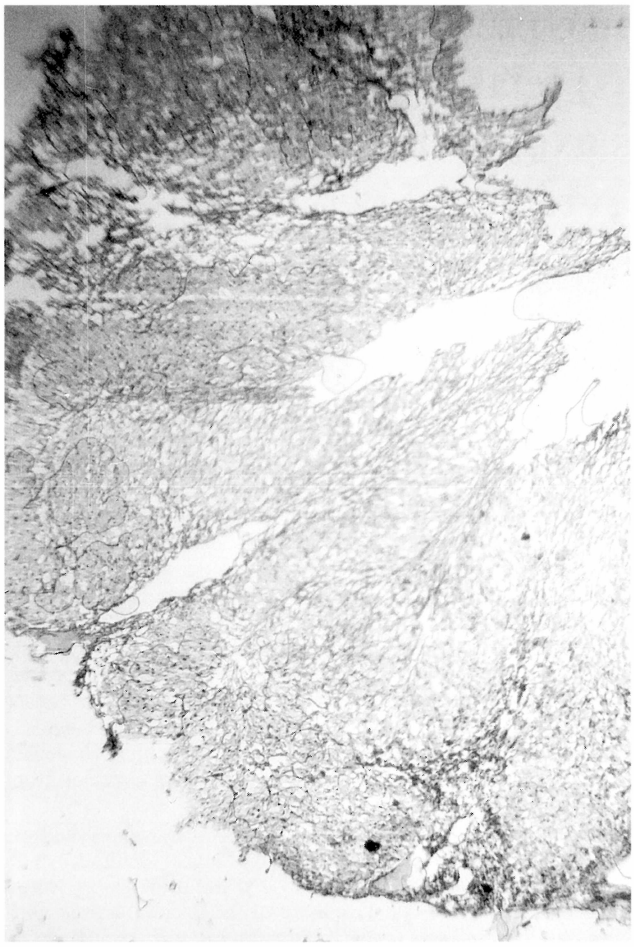
Methods

Two patients suffering from advanced cancer were taken as subjects for the present study on fetal thymus transplantation programme after getting informed consent from the patients and approval from the ethical committee. Following standard antiseptic, aseptic and premedication protocols, the patients were injected with 1% lignocaine infiltration anaesthesia at the proposed site of transplantation in the axillary fold of the skin of the recipients. A 1 cm long and 1 cm deep tissue space with good vascularity was dissected and prepared in each case to receive the thymic graft. At an adjacent table, a fetus was collected from a consenting mother undergoing hysterotomy and ligation (before 20 weeks) under general anaesthesia, for each case. Within a minute of hysterotomy, the thymus was dissected out from the fetus and transplanted at the prepared site in the subcutaneous space of the axilla and the skin was closed by (00 vicryl) atraumatic interrupted sutures. Haematological para-

Fig. 1



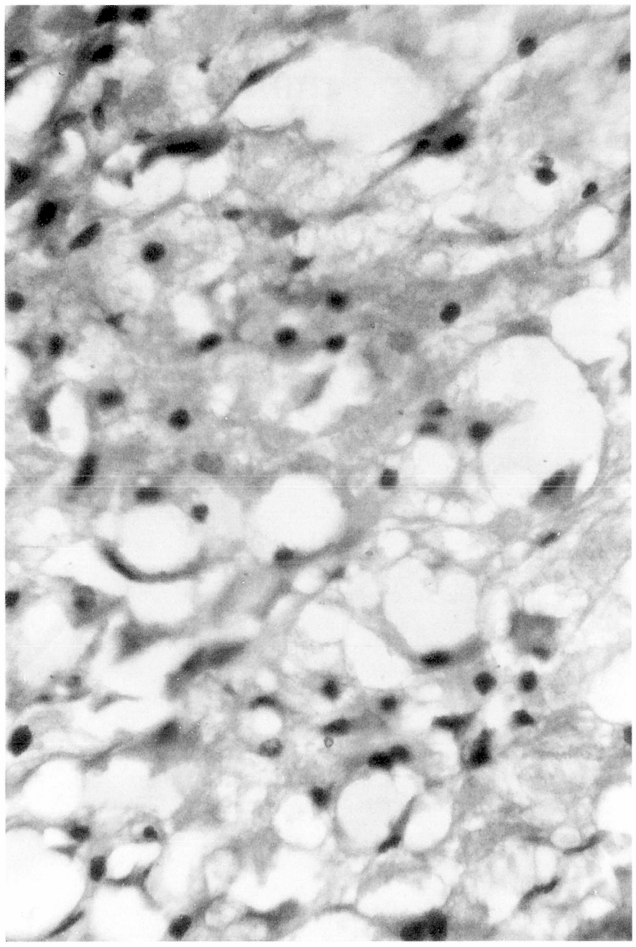


Fig. 2

Figures 1, 2. — Photomicrograph of a 16-week pre-transplant fetal thymus stained with haematoxylin and eosin. Figure 1 - Scan Power; Figure 2 - High Power.

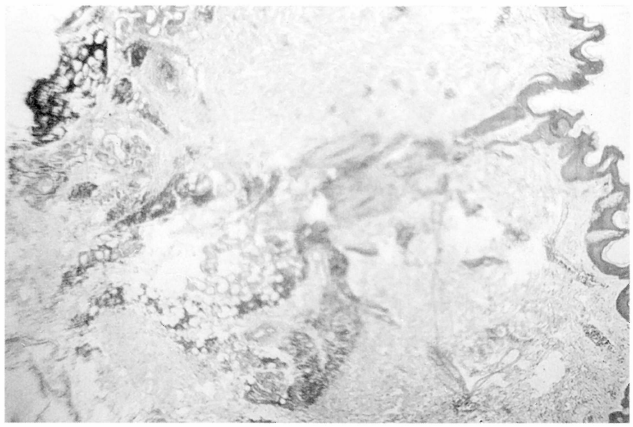
meters (Hb/Tc/Dc/ESR/platelets, etc.) were studied [11] in the preoperative phase and continued sequentially in the postoperative phase up to six weeks of the date of transplantation. After one month of the primary operation, the transplanted thymic tissue was taken out with an elliptical incision under local anaesthesia for serial histological evaluation. Skin suturing was completed with atraumatic 00 vicryl.

Case Reports

The first patient, Miss A.M. (14 years), had been suffering for five years with non-Hodgkins lymphoma (Ann Arbor Stage IV) and was receiving chemotherapy periodically with cyclophosphomide, vincristine, doxorubicin, and prednisolone after completion of her radiotherapy. However, the disease was showing features of progressive dissemination and there was an overall deterioration of the patient's condition. After getting proper informed consent from the patient's guardian and approval from the ethical committee, a 16-week hypo-immune (more than 15 weeks on the basis of the last menstrual period [LMP] calculation and ultrasound confirmation) human fetal thymus was transplanted in the patient. Preoperative haematological assessment showed haemoglobin: 13% gm, ESR: 45% mm/hr, peripheral blood total count (Tc) before the thymus transplant was 2,400/cmm with 28% neutrophils, 68% lympocytes, 2% each eosinophils and monocytes, no basophils were present and the platelet count was 75,000/cmm. On the seventh postoperative day of placement of the thymic graft, the Tc was 3,600/cmm with 36% neutrophils and 60% lymphocytes. On the 11th postoperative day, the WBC count rose to 9,400/cmm with 80% lymphocytes which became 86% with the total WBC count going up to 15,800/cmm on the 17th day of the transplant. This ascending trend of the WBC continued unabated and reached 33,000/cmm (86% lymphocytes) on the 23rd day and 42,000/cmm (88% lymphocytes without any blast cells) on the 30th day of thymic graft placement. The thymic graft was removed with the axillary adjacent tissue on the same day and on the seventh day of the removal of the human fetal thymic graft, the haematological assessment showed haemoglobin: 11.6% gm, ESR: 68 mm/hr, Tc: 9,900/cmm with 10% neutrophils, 86% lymphocytes, along with 2% each eosinophils and monocytes without any basophils. The platelet count was 110,000/cmm. The histology of the thymus after proper processing and staining with haematoxylin and eosin, when compared with the pretransplant same thymic tissue, showed growth and proliferation of the thymic tissue with Hassal's corpuscles, scattered and aggregated lymphocytes which showed comet-like structures. There was no endarterites, thrombosis or any other specific graft vs. host reaction feature.

The second patient, Mrs. P.M., 35 years old, was suffering from breast cancer of the right side (T_4, N_2, M_0 duct carcinoma) with negative oestrogen, progesterone and epidermal growth factor status, and was treated primarily with modified radical mastectomy and axillary clearance, with follow-up chemotherapy

Fig. 3



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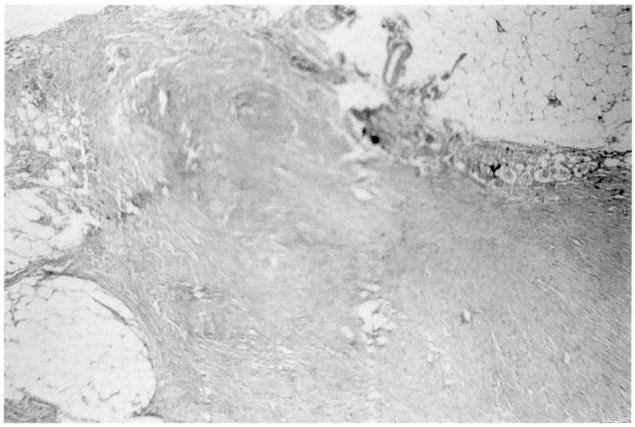

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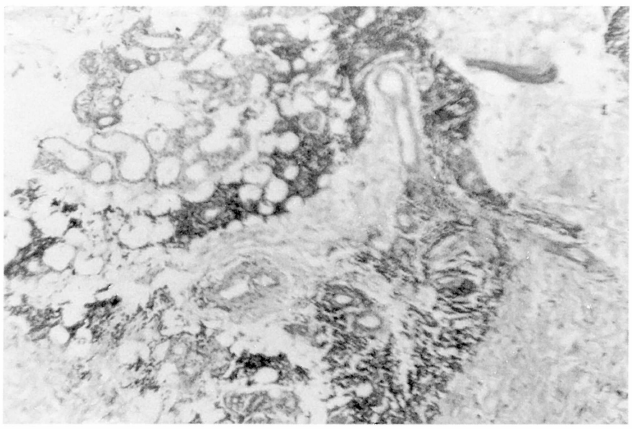
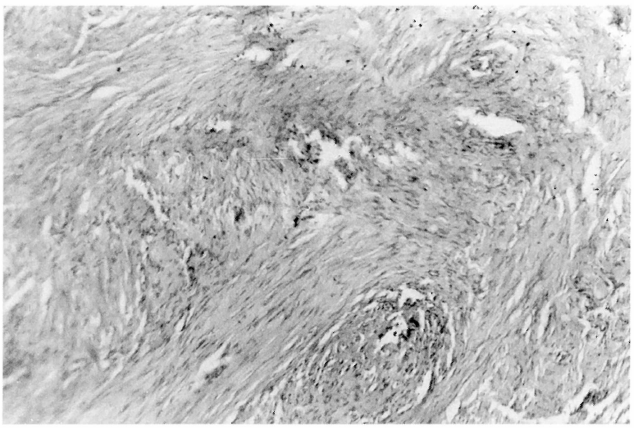


Fig. 7



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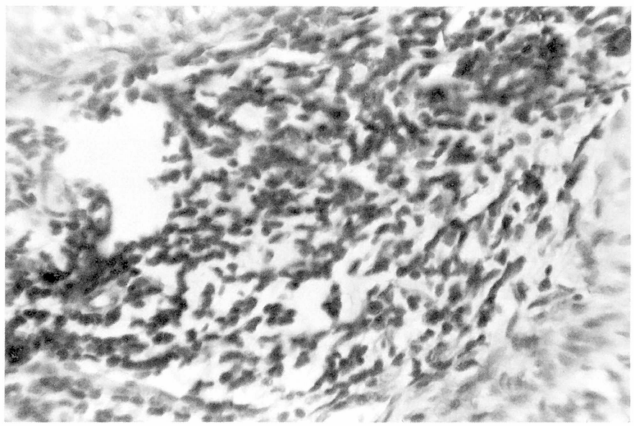
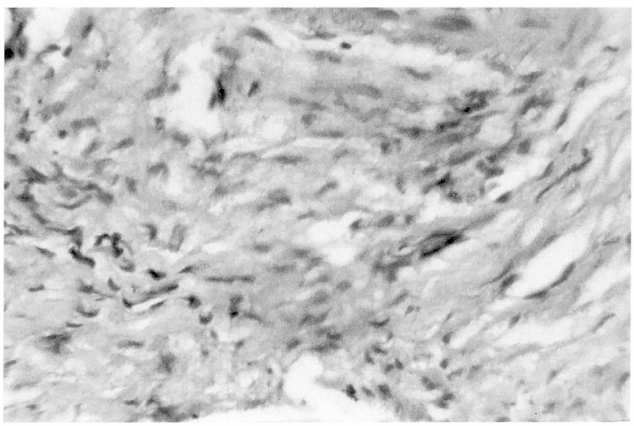


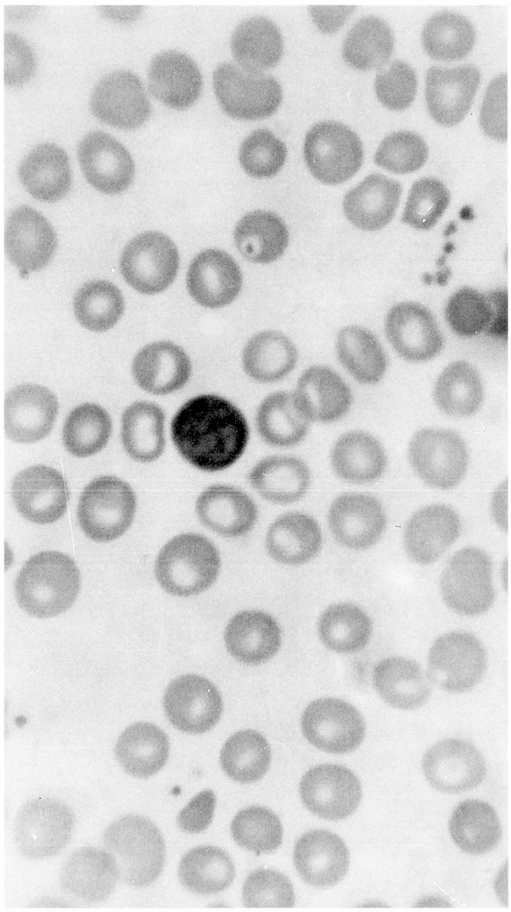
Fig. 8



Figures 3, 4, 5. — Photomicrograph of the transplanted fetal thymus (16 weeks) after four weeks of growth in a patient suffering from non-Hodgkin's lymphoma. Figure 3 - Scan Power; Figure 4 - Low Power; Figure 5 - High Power.
Figures 6, 7, 8. — Photomicrograph of a 12-week fetal thymus after one month's stay at the transplanted site in a patient suffering from breast duct carcinoma. Figure 6 - Scan Power; Figure 7 - Low Power; Figure 8 - High Power.

consisting of cyclophosphomide, methotrexate and 5-fluorouracil. This patient received a pre-immune (less than 15 weeks) tiny human fetal thymus (12 weeks) which was collected from a consenting mother undergoing hysterotomy and ligation. The graft was placed in the left axillary fold subcutaneous space under local anaesthesia. In this case, the preoperative haematological assessment showed haemoglobin: 12.6% gm with total WBC count at 8,500/cmm, with 70% neutrophils, 24% lymphocytes, 4% eosinophils, 2% monocytes, without any basophils, ESR: 45 mm/hr and platelet count 184,000/cmm. On the seventh postoperative day the total count became 8,600/cmm, with 72% neutrophils and 24% lymphocytes. The total count became 5,000/cmm on the 21st day with 64% neutrophils and 34% lymphocytes. The WBC count rose to 9,800/cmm with 74% neutrophils and 22% lymphocytes on the 23rd day. After removal of the thymic graft on the 30th day, the WBC count came down to 8,000/cmm on the seventh day with 68% neutrophils and 28% lymphocytes. The total count came down further to 6,250/cmm with 68% neutrophils and 30% lymphocytes on the 15th day. The haemoglobin count was 11.8% gm, ESR: 47 mm/hr, and the platelet count was 162,000/cmm on that day. Thymic histology revealed an appearance of Hassal's corpuscles, comet-like structures on the aggregated lympho-

Fig. 9



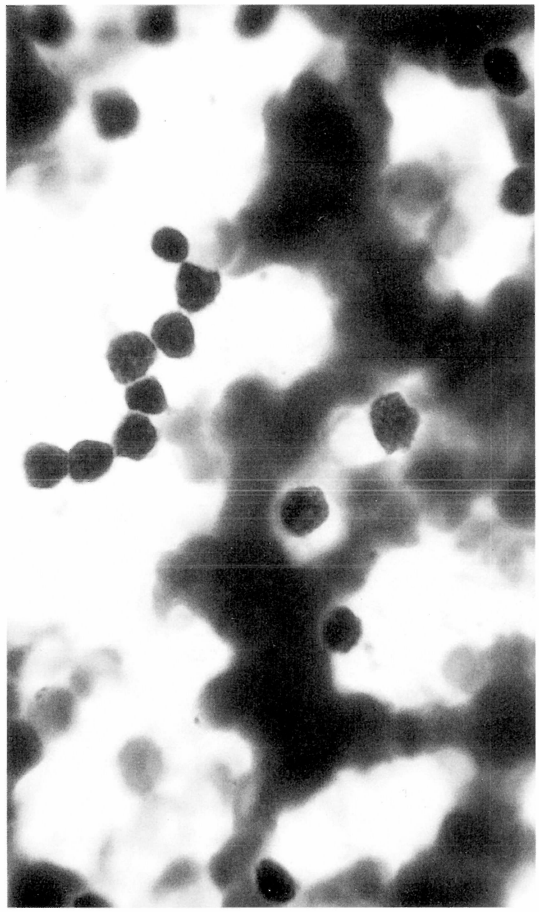


Fig. 10

Figures 9, 10. — Photomicrograph of peripheral blood leucocytes seen under an oil immersion lens of the microscope. Figure 9 shows the leucopenic phase of the pre-thymic transplant (16 weeks) slide in a patient with non-Hodgkin's lymphoma. Figure 10 shows the leucomoid impact of the thymic transplant in a patient with non-Hodgkin's lymphoma.

cytes, justifying proliferation and immuno-competence of the transplanted thymus in the background of sweat, sebaceous glands and squamous epithelium of the recipient's skin.

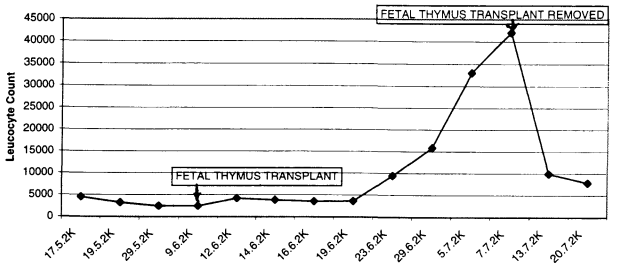
Discussion and Conclusion

The first point concerns the technique of thymus grafting – it has been shown by experiments that thymic fragments in which the structure is preserved are more effective than dissociated thymic cells in the restoration of functions [12]. If fragments of one thymus are placed in the muscle and compared with an injection of thymic cell suspension in another muscle, the surgically placed pieces would show better survival [13]. The present technique utilized the quasi-total human fetal thymus, 16 weeks old, from which a little tissue was taken out for histological comparison in the first case and the total thymus, 12 weeks old, was taken for the second case to avoid trauma and injury effects on this tiny thymic tissue (12 weeks). This sample method of human fetal thymus transplantation under local anaesthesia in advanced cancer patients without matching the HLA (human leucocytic antigen), has shown that the graft was not rejected at one month (period of observation). There was no graft vs. host (GVH) reaction noted clinically or histologically in either the case of non-Hodgkin's lymphoma (Case 1) or of breast duct carcinoma (Case 2). However, the pretransplant leucopenia was grossly overcorrected (leucomoid reaction) on the impact of a 16-week fetal thymus transplant and the leucocyte count returned to normal levels after the removal of the transplant in the case of non-Hodgkin's lymphoma, whereas the impact on the peripheral leucocyte count was less pronounced in the case of the patient with breast duct carcinoma, though the histological evidence of Case 1 and Case 2 both showed growth, proliferation and differentiation of the fetal thymus in the new host site.

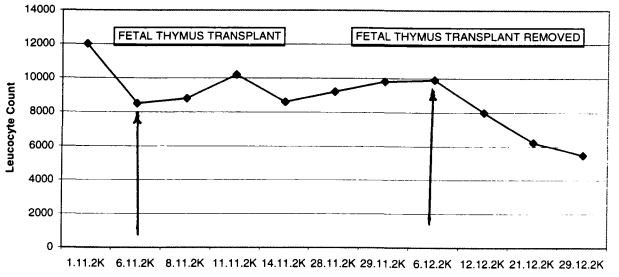
Fetal growth is dependent on a unique symbiotic environment where the mother provides all the necessary factors for the growth and differentiation of the growing fetal organs. The fetal micro-environment is distinctly different from the adult micro-environment [14]. It appears, therefore, that the developing fetal organ changes its own micro-environment in an altered metabolic situation by taking advantage of its hypo-immune and/or pre-immune status to survive, grow and differentiate. Harrison [15] has written an interesting review on the problem of surgery on the unborn where the advantages and disadvantages of pre-immune/hypo-immune fetal tissue surgery are discussed.

Graph 1.

EFFECT OF A 16-WEEK FETAL THYMUS TRANSPLANT IN A PATIENT WITH NON-HODGKIN'S LYMPHOMA AND LEUCOPENIA



EFFECT OF A 12-WEEK FETAL THYMUS TRANSPLANTATION IN A PATIENT SUFFERING FROM BREAST CANCER



The following graphs depict the impact of fetal thymic transplant on the leucocytes of the peripheral blood. Graph 1 shows the impact of thymic transplant (16 weeks) on non-Hodgkin's lymphoma and Graph 2 suggests the impact of thymic transplant (12 weeks) on breast duct carcinoma. Our observations on the above-mentioned cases are that thymic fetal tissue:

a) can grow and proliferate in cancer patients;

b) can violate the classical transplant concept of allogenic rejection of the graft (non-HLA matched) for at least one month (period of observation);

c) can even contribute to restoring the immune condition of the host, in case of existing deficiencies, for example, pretransplant leucomoid reaction, which returned to near normalcy after the excision of the 16-week fetal thymic graft.

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