Interruption of the Lymphatic Vessels and its Consequences in Total Homotransplantation of the Small Intestine and Right Side of the Colon in Man

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Summary

The problems of total lymphatic drainage interruption have been studied in a case of total homotransplantation of the small bowel and right colon with a clinical survival of 26 days. Marked stasis with distension of lymphatic tracts was noticed both in the mucosa of the colostomy and in the mesentery. At postmortem examination, no lymphatic regeneration was observed 26 days after grafting. Histological slides of the mesentery showed areas of oedema associated with pronounced lymphangiectasis. The lymphatic cavities were filled with red cells and the lymph nodes were apoplectic. The absence of lymphatic regeneration is a point of real interest, especially concerning both the secondary sclerosis owing to the lymphedema, that would compromise the function of the graft, and the absorption of fatty acids.

The absorption of these acids by the venous route either directly or indirectly through spontaneous lympho-venous anastomosis, in fact, seems to be a sufficient by-pass. Under these conditions, ligature of the lymphatics of the transplant is a safe procedure, thus preventing retro-peritoneal lymphorrhagia or chylous ascites. However, it would be perhaps useful to create such a lympho-venous anastomosis in order to diminish the stasis in the transplant until regeneration of lymphatics occurs.

The abundance of lymphatic tissue at the level of the intestine and the important role it plays in collecting and carrying away fat absorbed from the digestive mucosa suggested that interruption of the lymphatic vessels during intestinal transplantation might raise a number of special problems.

In the light of experimental results and our own findings following intestinal homotransplantation in man we shall attempt to bring our knowledge up to date in regard to the following five essential points:
– the immediate repercussions of raised lymphatic pressure on the graft;
– the late consequences of prolonged lymphatic stasis on the graft;
– the consequences of lymphatic interruption on absorption of fat;
– the possibilities of lymphatic regeneration in man;
– the possible immunological consequences of lymphatic stasis on the intestinal graft.

Case Report

Total orthotopic homotransplantation of the small intestine and ascending colon was carried out on January 22, 1969 in a 35 year old man with Gardner's syndrome with relapsing subacute occlusive phenomena. An exploratory laparotomy 9 weeks

previously had shown that the lesions were so extensive that complete resection appeared impossible and that ileostomy could not permit prolonged survival of the patient. Only the first jejunal loop could possibly be preserved, because polyposis of the colon was associated with fibromatous ileo-mesenteric tumors.

The donor and the recipient were both HL–A$_2$ in the first sublocus, in the second sublocus the recipient was positive for HL–A$_5$ and for the Da 2, 3, 6 and 7 antigens, whilst the donor was positive only for HL–A$_5$ and Da 6 and doubtful for Da 7. The patient's condition was very satisfactory for the first 10 days, but on the 11th day it suddenly deteriorated with the appearance of very severe mucous diarrhoea. On examination of the abdomen mild distension was found and a deep ill-defined and only slightly sensitive mass was palpated and this led to reoperation on the 14th day : the vascular and digestive sutures were perfect, the grafted intestine was normal in appearance but on palpation its wall was considerably thickened; peristaltic contractions were feeble and finally there was marked oedema of the root of the mesentery and examination of the aspirated fluid showed that it consisted of a bacteriologically sterile outflow of lymph. The patient's symptoms were then better interpreted and considered to be due to rejection of the graft. Immunosuppression, carried out until then with the association of azathioprine, soludecadron (Merck) and antilymphocyte serum, was then increased in dosage and azathioprine was replaced by injectable 6-mercaptopurine. The following 5 days the patient appeared definitely improved, but from the 19th day onwards his general condition gradually declined; the appearance of considerable leucopenia led us to stop the 6 mercaptopurine. The graft no longer appeared functional, as shown by measurement of digestion, but it was only during the last 2 days that the colostomy took on a necrotic appearance and the patient died on the 26th day with a clinical picture of toxic infection with hyperthermia and subclinical jaundice.

During grafting the mesenteric lymphatic vessels were ligatured using unasorbable, fine sutures (Tevdek 00000) both on the graft and on the recipient. The vessels had been rendered visible by the administration of 60 g of butter 4 hours before the operation. We may recall that the digestive continuity was ensured by an end-to-end jejuno-jejunal anastomosis, whilst the far end for the transverse colon of the graft was brought out onto the skin, in a barrel shaped anastomosis with the cut end of the recipient intestine. There was no leakage of lymph either in the retroperitoneal space, or in the abdominal cavity. On the other hand it is impossible to say whether some lymphatic transudate increased the outflow from the colostomy, which was on average between 1100 and 1200 cc of fluid per 24 hours.

As far as lymphatic stasis at the level of the intestine and mesentery was concerned this had important consequences:

a) at the level of the intestinal wall, stasis occurs immediately and is demonstrated by biopsy of the grafted jejunum during the operation. During the following days, comparison between the colostomy of the remaining colon in the recipient and that of the graft showed considerable oedema of the latter; on the biopsy of the colonic mucosa carried out on the 12th day, there was considerable lymphatic distension clearly visible in the sub-mucosa;

b) *at the level of the mesentery,* distension of the collecting lymphatics associated with outflow of lymph between the mesenteric folds was so great that it became palpable, giving the impression of a deep mass which led us to reoperate on the 14th day as they feared a perianastomotic abscess: in fact the samples removed at this level remained sterile;

c) *death occurred on the 26th day and finally it was the autopsy* which provided the main information:

- *Macroscopically,* the intestinal loops were distended, open and rigid with thick, grey walls. The mesentery was thickened (3 cm) and filled with large, black, infarcted lymph nodes (Fig. 1). The mesenteric changes suddenly ended without any transitional stage, on the line of reinsertion and the peripancreatic area of the recipient appeared little changed. The mesenteric vessels were permeable throughout their course; the digestive and vascular sutures were intact.

- *Histologically,* the mucosa of the whole graft was torn and replaced by large haemorrhagic and necrotic areas. The sub-mucosa was very thickened and dissociated by considerable oedema mixed with areas of erythrodiapedesis; the sub-serosa also appeared very thickened.

At the level of the sub-mucous lymphatic plexuses there were marked areas of lymphangectasia (Fig. 2) and the sections of the jejuno-jejunal suture clearly showed the difference with the recipient: immediately above the scar, which was not crossed by the sub-mucosal lymphatics, the latter appeared almost normal, were simply a little dilated as is usual during congestion. The lymphatic changes were definitely the histological expression of the parietal thickening of the grafted small intestine and colon and the second important fact was the presence, in spite of the relatively limited
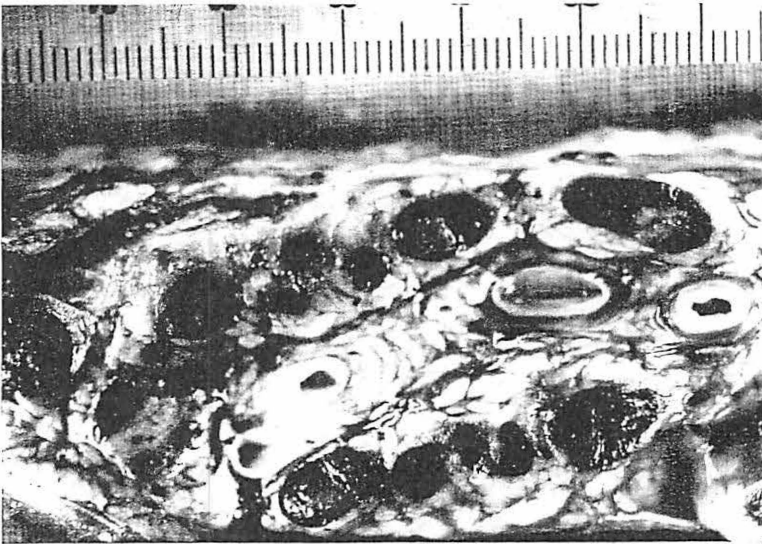

Fig. 1 Section of the mesentery showing considerable thickening and numerous necrotic lymph nodes.

character of the survival, of early fibrous organisation; observed mainly at the level of the colonic mucosa, fibrosis of the lamina propria surrounded in some areas by islets of cells in regression. There were also signs in the sub-mucosa and sub-serosa, the connective tissue there being represented mainly by mesenchymatous tissue with few fibrils.

The changes in the mesentery were the third main element: there were large areas of oedema and haemorrhage, separated by fibrous septa of variable thickness with liponecrotic foci surrounded by lipophagic masses. The lymphoedema was particularly marked in contact with the suture line where the root of the host mesentery contrasted with the almost normal appearance of the peripancreatic region and preaortic region of the recipient, this suture area also contained fibrin deposits. On the sections were noted marked lymphangectasia, distributed in bundles; some, mainly in contact with the ganglia, were filled with red blood cells. On elective staining for reticulin one may show the presence of breaks in the wall of the distended lymphatic vessels; they cause true "lymphorexia" with lymphorragia, the mechanism of which is undoubtedly mechanical (Fig. 3). As far as the mesenteric lymph nodes are concerned, they are very large and are the site of haemorrhagic necrosis. One may find only a few islets of lymphoid tissue surrounded by large apoplectic areas. The efferent lymphatics are also often engorged with red blood cells. The presence of red blood cells within lymph nodes may be explained by phenomena of massive haemorrhagic necrosis affecting the mucosa and followed by evacuation of red cells through lymphatic ducts and accumulation within the nodes; these facts do not however take into consideration the appearances of infarction observed in these ganglia (Fig. 4). We believe that these are true immune infarctus due to vascular necrosis in the corresponding area.
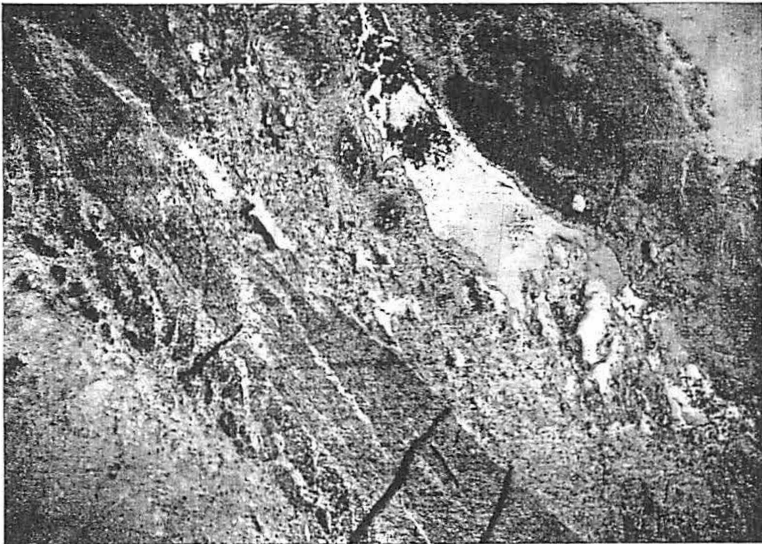

Fig. 2 Very dilated lymph vessel below the ulcerated mucosa: It contains lymph and numerous red blood cells.

Fig. 3 Tear in the wall of a very distended lymphatic duct in the mesentery. The contained lymph appears continuous with the oedema of the poorly vascularised connective tissue.
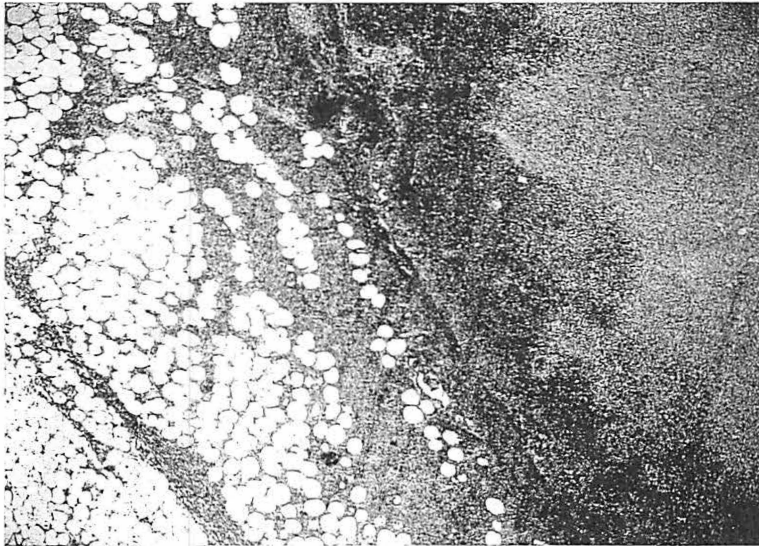

Fig. 4 Mesenteric lymph node; The lymphoid tissue persists only on the periphery forming a "border", whilst most of the lymph node has undergone infarction.

Discussion

This case report is the first intestinal human homotransplantation, that was performed for Gardner's Syndrom[1]. Two intestinal grafts were performed previously (6, 7), but after massive résections for total infarction of the territory of the superior mesenteric artery. The consequences of interruption of the lymphatic vessels were not studied (the patients survived 2 and 12 days respectively).

For many reasons experimental homotransplantation carried out in the dog gives little information on lymphatic vessels. Firstly, the rejection phenomena have mono-polised interest and on the whole have not permitted sufficiently long survival of either the recipient or the graft, thus no study of lymphatic regeneration following orthotopic homotransplantation in man has yet been published. In particular *Kocandrle* (5) in 14 dogs did not observe any case of survival beyond the 9th day. Only in autotransplanted dogs this author was able to show the reconstruction of a lymph flow by lymphography, from the 20th day onwards, with passage of the opaque substance (Ethiodal) in the thoracic duct.

Furthermore the great majority of publications concern intestinal grafts in the neck which are very far from physiological conditions, especially in regard to the surrounding environment, the small length of the transplant and the absence of any food bolus. The studies of *Preston* (10), *Slaney* (11) and especially *Grenier* et al. (4), which are most remarkable, lead to three conclusions:

1. Lymphatic stasis occurs very early and is very marked in the form of oedema, distension of the lymphatic sinuses and major hypertrophy of the mesenteric lymph nodes; its late repercussions cannot be validly assessed for phenomena of rejection interfere too rapidly;

2. Regeneration of the lymphatic vessels has never been noted, either because the animals die too soon, or because the graft becomes involuted or necrotic due to rejection; its average functional survival time during treatment with immunosuppressive agents is roughly 6 to 18 days;

3. The absorption of certain fats is possible: *Grenier* et al. have shown that the more effective is the immunosuppressive treatment, the higher the enzyme levels measured in the intestinal juice and the higher the intestinal absorption of C 14 labelled oleic acid and trioline through the intestinal graft. This absorption which in the protected animal is roughly 10% may be seen early when there is obviously not yet any lymphatic regeneration.

There are two possible explanations. Firstly there may be intertissular repermeabilisa-tion between the graft and the surrounding tissues as injection of methylene blue into the intestinal wall is sometimes followed, at a later stage, by traces of methylene blue in the neck tissues; this phenomenon which is special to the experimental method adopted obviously does not permit any extrapolation for human orthotopic homografts. Further-

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[1] Since the preparation of this paper another intestinal graft for Gardner's Syndrom was performed by *J. G. Fortner* (New-York): the patient survived some ten weeks (personal communication, 1971).

more, and above all, it is probable that fatty acids are absorbed through the blood as may be noted physiologically with certain short chain triglycerides and also the opening up of lymphovenous anastomoses is no doubt possible.

In our case most of the changes observed are clear evidence for the absence at the end of the survival period of 26 days of any sort of lymphatic restoration, either at the level of the mesenteric suture or at the level of the intestinal suture. They are mechanical in nature and lead first to lymphoedema which is the responsible explanation for the thickening of the mesentery and of the intestinal walls. Transudation, linked to intra-lymphatic high pressure, is associated with many partial breaks in the collecting lymph vessels, responsible for true lymphorrhagia mainly into the mesentery.

These early changes lead one to consider the late effects on graft function of prolonged lymphatic stasis. As suggested by human clinical experience, it is liable to induce the appearance of fibrosis, with its serious consequences. In our case, the latter was already present in the mucosal septa, the sub-mucosa and the mesentery.

The technical possibilities of reducing this lymphatic stasis are small; this stasis is certainly less serious than with the chylus ascites which one would obtain if the lympha-tic vessels were not tied off; the only technical possibility would be a lymphovenous anastomosis at the level of the mesentery.

The absorption of fat does not seem to raise any problems, provided lymphatic regeneration occurs later, as in any case the loss of the villi seems to last longer than the absence of lymphatic circulation and it is the presence of a functional mucosa which is the primary condition of fat absorption. In the meantime, the opening of lymphovenous anastomoses and above all physiological absorption of certain fats through the venous route will occur.

By analogy with what is known in lymphoedema of the limbs and taking into con-sideration the fact that the intestinal contents are not sterile, it is probable that lymphatic stasis has a harmful effect on the intestinal flora. The upset which it may cause is ob-viously favoured and aggravated by the immuno suppressive treatment. Finally, des-quamation of the mucosa is a major source of infection and all these factors together explain the extremely severe necrosis which occurs on the grafted intestine.

As far as the immunological problem is concerned, our object is not to describe here in detail the results of interaction between the two incompatible lymphatic populations; in our transplantation there was in the second sublocus the possibility of a graft versus host reaction and not a reaction of the host against the graft. However, there was mainly a recipient-graft rejection with lesions of immune infarct type at the level of the mesen-teric lymph nodes, whilst the reaction of the graft against the host was mild; this occurred before any lymphatic repermeabilisation and thus drainage of the thoracic duct, as suggested by *Okumura,* seems quite useless. It is obvious that the antigenic information and its immune response could only occur by the blood route, which confirms the demon-stration by *Coulic* and *Govallo* (3), of humoral lymphocytotoxic and haemaglutinating antibodies during intestinal homotransplantation in the neck. The lymphatic circulation thus seems to have only a secondary role and may be totally supplanted by the blood flow.

Addendum:
  Since the preparation of this manuscript the following publication relating to the present case has been published:

Alican, F., J. D. Hardy, M. Cayirli, J. E. Varner, P. C. Moynihan, M. D. Turner, P. Anas: Intestinal trans-plantation: Laboratory experience and report of a clinical case. Amer. J. Surg. 121 (1971), 150-159

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Lymphatic Dissemination of Cancer Cells in Mice Transplanted Intratibially with Ehrlich Carcinoma*

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Summary

Ehrlich carcinoma cells implanted in the marrow cavity of the tibia in Swiss mice induce the formation of visible metastases in axillary lymph-nodes. The pattern of the lymphatic vessels in the tumor implantation area was investigated. Cancer cells were found in lymph samples taken from the subcutaneous inguino-axillary lymphatic trunk. The blockade of this lymphatic vessel by a polyvinylic resin resulted in a different distribution of lymphnodal metastases, while the hematic dissemination of cancer cells was unaffected. The results are discussed in relation to the pathogenesis of the axillary metastases in the considered experimental model.

Previous studies (1) indicated that Ehrlich carcinoma implanted in the marrow cavity of the tibia in Swiss mice induced the formation of visible metastases in distant lymph-nodes such as axillary, paraortic, thoracic, paravertebral, and cervical nodes. These findings suggested a study to identify the lymphatic circulation in the posterior leg of the mouse after the intratibial transplantation of Ehrlich carcinoma and to analyse the distribution of lymphnodal metastases after inducing lymphodynamic changes.