

## SELECTED ABSTRACTS OF PAPERS PRESENTED AT THE 10TH INTERNATIONAL CONGRESS IN ADELAIDE

### BENZOPYRONES AND POST-MASTECTOMY LYMPHEDEMAS DOUBLE BLIND TRIAL PLACEBO VERSUS SUSTAINED RELEASE COUMARIN WITH TRIOXYETHYLROUTIN (T.E.R.)

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Open trials carried out by Clodius and Piller have shown the beneficial effect of the benzopyrones on post-mastectomy lymphedemas. Our trial included 91 subjects divided into 2 groups by drawing lots (active product — placebo) and treated 6 months at a daily dose of nine 15 mg sustained release coumarin tablets with T.E.R., followed by a 18 month period of 6 tablets/day of active product.

The results have shown: after 6 months, a decrease of the measurements in comparison with the healthy side in the group treated with active ingredient and an increase in the control group. This difference was statistically significant in respect to the wrist

( $p < 0.02$ ), the forearm ( $p < 0.01$ ) and upper arm ( $p < 0.005$ ). In the group treated with active ingredient, this decrease was continuing at the 12th and 18th months (wrist  $p < 0.01$ , arm  $p < 0.04$ ).

Isotopic lymphography showed, after 6 months, a decrease in the number of patients with blockage in the axilla, in the group treated with active ingredient ( $p < 0.03$ ). Tolerance was considered to be excellent, with 10 cases of minor side effects in the group treated with active product, and 2 cases in the control group.

This controlled trial supports the efficacy of the benzopyrones in the treatment of post-mastectomy lymphedemas.

### RETROPERITONEAL LYMPH NODE DIFFUSION IN ENDOMETRIAL CARCINOMA: IS LYMPHOGRAPHY ADEQUATE FOR STAGING?

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The availability of a group of 1055 clinically assessed FIGO stage I patients with carcinoma of the endometrium provided significant data about the parameter "lymph node involvement" as a prognostic indicator and possible major condition influencing survival. All the patients had surgery and in 669 the histologic results of selective or systematic retroperitoneal lymphadenectomies were available. Lymph node metastases were proven in 37 (5.8%).

From our case material the following results in regard to the influence of the so-

called prognostic factors on the percentage of lymph node metastases were obtained: a) when the *grade* of the tumor was considered, the incidence of N+ was 3% for G1, 8% for G2 and 13% for G3. b) when *myometrial invasion* was the parameter, the chance of having nodes involved was 0% for M0, 2% for M1, 5% for M2 and 21% for M3. c) considering *vascular invasion*, it was absent in 96.7% if lymph nodes were normal, while present in 26.5% when involved.

Lymphography was applied in a consistent number of patients and radiographs are

under review by an expert committee. The results of the first 284 cases were: accuracy 96.9%, sensitivity 31.2% and specificity 96.6%. Histologically about a half of the metastases were less than 5mm in diameter, thus impossible to detect lymphographically: if we exclude these cases, sensitivity ranges up to 55.5%.

Each prognostic factor influences the percentage of nodal involvement and these metastases strongly condition survival. Unfortunately lymphography seems to be inadequate for an accurate detection of adenopathies and for this reason lymphadenectomy appears necessary.

## **ENDOLYMPHATIC APPLICATION OF LIPOSOMES CONTAINING BLEOMYCIN IN RABBITS**

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Successful endolymphatic therapy with cytostatic agents requires lipoidal carriers. Such carriers permit cytostatic agents to be transported via the lymphatic vessels and to be deposited in the lymph nodes. In this report, the preparation and application of liposomes containing a cytostatic agent is described.

Pure liposomes were formed by dialysis of 100mg egg yoke lecithin and 100mg n-octylglucoside in 8ml of an isotonic phosphate buffer pH 7.4. Subsequently, bleomycin was incorporated into the pre-formed liposomes by method of Schubert (unpublished data). These bleomycin-containing liposomes were stable, unilamellar, and homogenous with an average diameter of 180nm. The preparation could be concen-

trated by ultracentrifugation, and used in the experiments with rabbits.

Rabbits received 2.5ml of the liposome suspension in the popliteal lymph node over a period of 30 min. Subsequently the distribution of bleomycin among several organs was determined. With the exception of the site of the injection, the highest concentration of bleomycin was found in the retroperitoneal lymph nodes, the intended targets.

In the typical liposome-storing organ (liver and spleen), up to a 25 fold lower bleomycin-level was observed.

These findings can have clinical relevance. The coating of such liposomes with anti-tumor monoclonal anti-bodies could greatly increase the effectiveness of drug delivery by this method.

## **INDIRECT LYMPHOGRAPHY IN LYMPHEDEMA**

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Subepidermal infusion of the non-ionic water soluble contrast medium Iotasul (Schering, Berlin) leads to an opacification of initial lymph vessels in the skin and of peripheral collectors.

114 patients with various forms of lymphedema were studied. In patients with primary lymphedema (n=50) four patterns of findings were differentiated:

1. Local aplasia of initial dermal lymphatics in the presence of abnormal sub-

cutaneous collectors (7/50).

2. Net-like hyperplasia of initial lymphatics with a reduced number of peripheral collectors was seen in most patients with lymphedema praecox and tardum (28/50).

3. Hyperplasia of initial lymphatics and of collectors was seen mainly in patients with a congenital lymphedema of the whole leg (9/50).

4. In six cases with severe sclerosis of the skin no definite lymph vessels could be

demonstrated.

From a practical standpoint indirect lymphography seems to be a useful method especially for the diagnosis of mild lympho-

edema and for excluding patients with hypoplasia of initial lymph vessels from surgery.

## CHARACTERISATION OF LYMPH FLOW BY SIMULTANEOUS AND CONTINUOUS MONITORING OF DEPOT CLEARANCE AND NODAL UPTAKE USING TC99M LABELLED COLLOID

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Previous efforts at measurement of skin lymph flow have employed an isotope clearance technique using  $^{131}\text{I}$ -Human Serum Albumin but this method has never been fully evaluated. Recent advances in nuclear medicine have established the value of  $^{99\text{m}}\text{Tc}$ -labelled colloids for imaging of the lymphatic system, so we have attempted to characterise lymph flow dynamically by simultaneous and continuous monitoring of depot clearance and nodal uptake of  $^{99\text{m}}\text{Tc}$  labelled colloid.

Five normal subjects were studied with each limb examined sequentially. 9.93mls of  $^{99\text{m}}\text{Tc}$  labelled rhenium sulphide colloid (TCK 17, C15) with an activity of 20-30 uci were injected superficially in the dermis of the dorsum of the foot. Basal lymph flow was established over one hour with the subject resting horizontally. Massage to enhance lymph flow was performed for a further 30

minutes. A linear monoexponential clearance of colloid from the injection site was demonstrated. Basal lymph flow was calculated as a half clearance time ( $T_{1/2}$ ). Massage produced faster lymph flow ( $p < 0.001$ ). Lymph flow was calculated from nodal uptake as the rate of rise in radioactivity ( $\text{counts sec}^{-2}$ ). A significant increase in nodal activity following massage indicated enhanced lymph flow ( $p < 0.001$ ).  $T_{1/2}$  correlated with nodal uptake. This technique characterises limb lymph flow including skin lymph flow as judged by two parameters — injection site clearance and nodal uptake. A highly significant increase in lymph flow by massage (effleurage) was demonstrated which confirms the benefit of this procedure for stimulating lymph flow.

In lymphedema where injection site clearance appears to be normal nodal uptake is markedly reduced.

## COMPUTED TOMOGRAPHY IN THE DIAGNOSIS OF LOWER EXTREMITY EDEMAS

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In *lymphostasis* CT scans demonstrate clear swelling of homogeneous subcutaneous fatty tissue and the muscle mass is distinctly identifiable. In *venous stasis* the subcutaneous tissue is inhomogeneous and slightly swollen. The borders of severely swollen muscles are irregular and unclear.

Nine patients with unilateral lymphostasis, two with acute venous thrombosis and 4 with the postthrombotic syndrome

were compared with 15 normal legs taking 8mm thick CT slices exactly from the same part of legs. Using the Siemens Somatome 2's own computer programme, the total area, the subcutaneous and muscle areas in slices were compared. These areas are roughly correlated with the volumes of each tissue.

*Results:* In addition to the visual description above there were changes in the volumes of the different tissues. In the following the

increase in different tissue areas in CT slices are compared to the total increase of the transection area.

In *lymphostasis* the subcutaneous tissue area increased  $85\% \text{ SD} \pm 12\%$  (ranges 65% to 100%) but the muscular tissue area increased only  $5\% \text{ SD} \pm 13\%$  (ranges -20% to +30%). In *acute venous thrombosis* the subcutaneous tissue area increased only 30% but the muscle area enlarged 60%. In the *post-*

*thrombotic syndrome* in which both veins and lymphatics are compromised the differences are less distinct: the subcutaneous area increased 60% and the muscle area 30%.

*Conclusion:* In the early phases of leg edema a CT can easily differentiate lymphatic and venous stasis but in the later phase it reveals that components of both types of stasis exist in edematous legs.

## HISTOLOGICAL EVALUATION OF THE CRURAL LYMPH NODES IN THE PRIMARY LYMPHEDEMAS OF THE LOWER LIMBS

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Application of microsurgical techniques in the treatment of the primary lymphedemas gave us the occasion to handle directly the lymph nodes and collectors of the diseased limb. In the last 46 cases of primary lymphedemas of lower limbs treated with lymphaticovenous shunts, the lymph nodes and afferent and efferent collectors were biopsied and submitted for histology.

In all lymph nodes examined, fibrosclerosis was uniformly found independent of the age of onset or duration of the

lymphedema. Afferent collectors demonstrated patency of the lumen and in 83% of the cases prominent fibromuscular hyperplasia of the wall while efferent collectors were normal or dilated.

This histological study together with the good results obtained with lymphatico-venous shunts that bypass the lymph node, seem to confirm the hypothesis of the primary role of fibrosclerotic lymph nodes in the pathogenesis of primary lymphedemas of the lower limbs as suggested by Kinmonth and associates.

## ESTABLISHMENT OF A HUMAN ENDOTHELIAL CELL LINE (CH3) FROM A RECURRENT RETROPERITONEAL LYMPHANGIOMA

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In contrast to blood vascular endothelium, the morphologic, immunologic, and biochemical properties of lymph vascular endothelium remain poorly characterized due in part to lack of comparable endothelial cell lines for in vitro study. Based on our earlier success in identifying discrete endothelial cell subpopulations in a mixed explant culture of a giant cervicodiastinal cystic hygroma, we undertook isolation (200U/ml Type IV col-

lagenase) and characterization of endothelial cells in monolayer from a recurrent, massive, chylous, retroperitoneal lymphangioma. Histologically, the resected tumor showed a complex structure with irregular cystic spaces intertwined with fibroadipose tissue, smooth muscle, and nodular lymphoid aggregates. The cyst lining was positive for endothelial marker Factor VIII-related antigen with immunofluorescence microscopy. From the

tumor, a homogeneous polygonal cell line has been successfully carried in continuous culture for 10 months. Scanning and transmission electron microscopy of the cultured cells showed a relatively smooth surface with variable microvillar projections, bundles of intermediate filaments, Weibel-Palade bodies, dilated rough endoplasmic cisternae, numerous vesicles and Golgi intracellularly, and also deposition of extracellular matrix.

Fluorescent antibody labeling of the cell line revealed Factor VIII-related antigen and cell surface fibronectin. Thus, this endothelial cell line derived from a rapidly growing lymphatic vascular tumor resembles both normal lymphatic endothelium and lymphangiomatous cyst lining morphologically and immunologically and thereby provides a new source of endothelial cells for future study.

## POSSIBLE DIETARY REGULATION OF LYMPHOCYTE TRAFFIC THROUGH INTESTINAL LYMPH

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Lymphocyte traffic through intestinal lymph is important for the maintenance of the population of plasma cells in gut mucosa. However little is known about the factors that regulate the migration of lymphocytes. Recent studies have shown that several intestinal functions can be modified by diet in the lumen. Therefore, we conducted this study to examine the influence of diet on flow and lymphocyte traffic of intestinal lymph.

### MATERIALS AND METHODS:

Mesenteric lymphatic duct of male Wistar rats (300g) was cannulated to collect intestinal lymph continuously. Lymph flow and lymphocyte number were measured at intervals and the effect of lipid (olive oil or micellar solutions of linoleic acid) and carbohydrate (different concentration of glucose solutions) administration on these parameters were measured. In another set of experiments, in vivo microscopy was used for the direct observation of lymphocytes in collecting lymphatics

of mesentery. Lymphocyte subpopulations were characterized using anti-rat lymphocyte monoclonal antibody (OX6 & W3/13).

**RESULTS AND CONCLUSION:** More than 99% of cells in intestinal lymph were lymphocytes and the dominance of IgA containing cells in intestinal lymph was characterized in lymph, T-cell populations (W3/13 positive cells) were greater than B-cells (OX6). Infusion of olive oil or micellar solution induced increased lymph flow and lymphocyte transport through intestinal lymph. Glucose infusion also stimulated lymph flow as well as numbers of lymphocytes. However, the effect of glucose was transient while lipid infusion was more prolonged. But, in both lipid and carbohydrate infusion, T/B cell ratio of intestinal lymph were not significantly altered. These results suggest that dietary stimulation might be one factor regulating lymphocyte traffic in intestinal lymph.

## EXPERIMENTAL MICROVASCULAR TRANSPLANTATION OF LYMPH GLANDS TO RESTORE LYMPHATIC FUNCTION

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Microvascular lymph node transfer to the lymphedematous limb to improve drainage, using a canine model of lymphedema below the knee was studied. The model was created with preliminary irradiation followed by skin stripping and then ablation surgery of the lymphatics.

A superficial inguinal lymph node on the normal side was removed with its vascular supply and perilymphnodal tissue, and transferred to the affected leg to bridge the obstructed knee area. Because this superficial inguinal lymph node drains only the buttock and lateral thigh, its removal does not affect drainage of the normal limb. One group of dogs had the proximal lymphatics of the transfer sutured to lymphatics of the limb but not the second group. In the second group

no anastomoses were formed.

Technetium 99 sulphur colloid scanning is a noninvasive method used to study the course of regeneration of lymphatics and to evaluate the function of the transferred lymph node.

Lymphangiograms proved the reestablishment of lymphatic continuity from distal limb through the transferred lymphatic tissue to the proximal lymphatics. The improved lymphatic drainage did make the affected limb smaller. The decrease of limb size was gradual and progressive throughout the six months of observation. The improved lymphatic drainage is also demonstrated by radioactive scanning. Both groups give comparable results.

## THE EFFECTS OF LYMPH NODE EXTIRPATION ON LYMPH FLOW

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The composition of lymph is used to give an indication of conditions prevailing in the tissue from which the lymph is collected. Because of the relative sizes and numbers of lymph ducts, it is difficult in many situations to cannulate vessels afferent to a lymph node for long-term collection of lymph. Therefore lymph is usually collected from the efferent duct of the regional node draining a tissue, despite the fact that its composition might be changed considerably on passage through the node. To overcome this problem, some studies have been done on the effects of extirpation of lymph nodes on the flow and composition of lymph from their drainage area. Prescapular lymph nodes of cattle were extirpated by incising the lymph node capsule and shelling out the node parenchyma. The incision in the empty capsule was then repaired with fine sutures. At variable periods from 6 weeks to 1 year after extirpation, lymph ducts have been sought in the area of the extir-

pated nodes. In each case so far a large single vessel has been found, corresponding to the efferent duct of the extirpated node, and cannulated. The lymph collected from these ducts has been clearly peripheral in nature, with many veiled macrophages which are usually found only in peripheral lymph. In some animals the efferent duct from the contralateral prescapular node was cannulated at the same time. The rate of flow of lymph from the vessel on the side from which the node was removed was similar to, or slightly less than that from the intact node. Peripheral lymph from the drainage area of extirpated nodes has flowed for up to 4 weeks at flow rates of up to 50ml/h. These results indicated that it is possible to study large amounts of lymph unaltered by passage through a node. They are also relevant to the serious untoward sequelae of lymph node ablation in human patients.