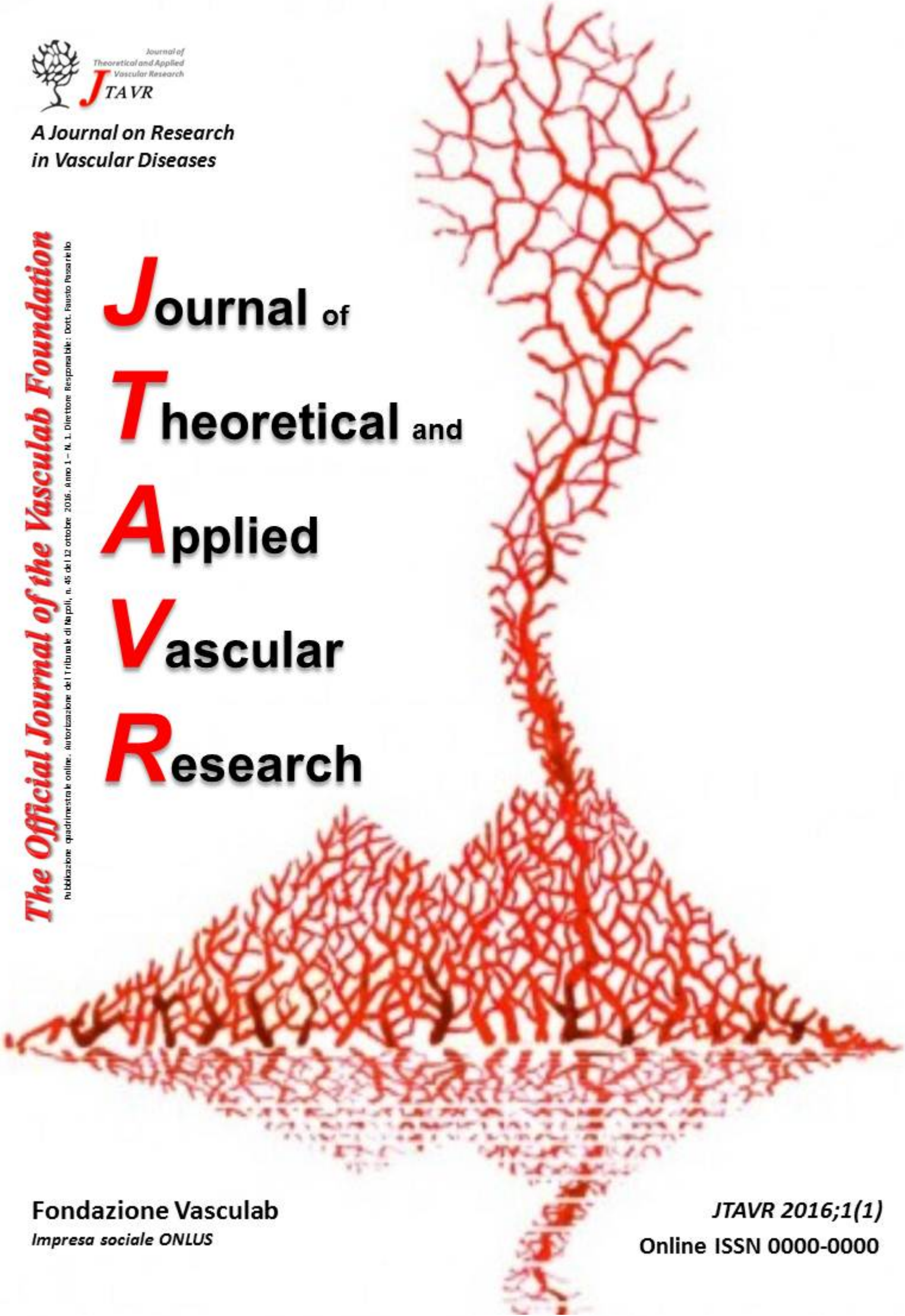
Journal of Theoretical and Applied Vascular Research
JTAVR

A Journal on Research in Vascular Diseases

The Official Journal of the Vasculab Foundation

Pubblicazione quadrimestrale online. Autorizzazione del Tribunale di Napoli, n. 45 del 12 ottobre 2016. Anno 1 – N. 1. Direttore Responsabile: Dott. Fausto Passariello

Journal of Theoretical and Applied Vascular Research



Fondazione Vasculab
Impresa sociale ONLUS

JTAVR 2016;1(1)
Online ISSN 0000-0000

International School of Vascular Ultrasound
VASCULAB, the Vascular List!

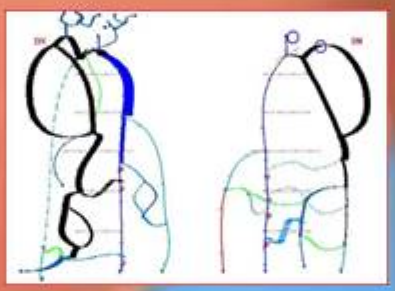
MEDISOL
medical solutions.

Corso di Aggiornamento

22 Febbraio 2017 - Napoli (NA)

EcoColorDoppler

Emodinamica Venosa



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N. 01-2016, year 1

Journal of Theoretical and Applied Vascular Research

A Journal on Research in Vascular Diseases, published three times a year

The Official Journal of the Vasculab Foundation

Editor

Fondazione Vasculab impresa sociale ONLUS, Via Francesco Cilea, 280 - 80127 Napoli - Tel/Fax +39 081 7144110 - jtavr@vasculab.eu - www.vasculab.eu/jtavr.xml

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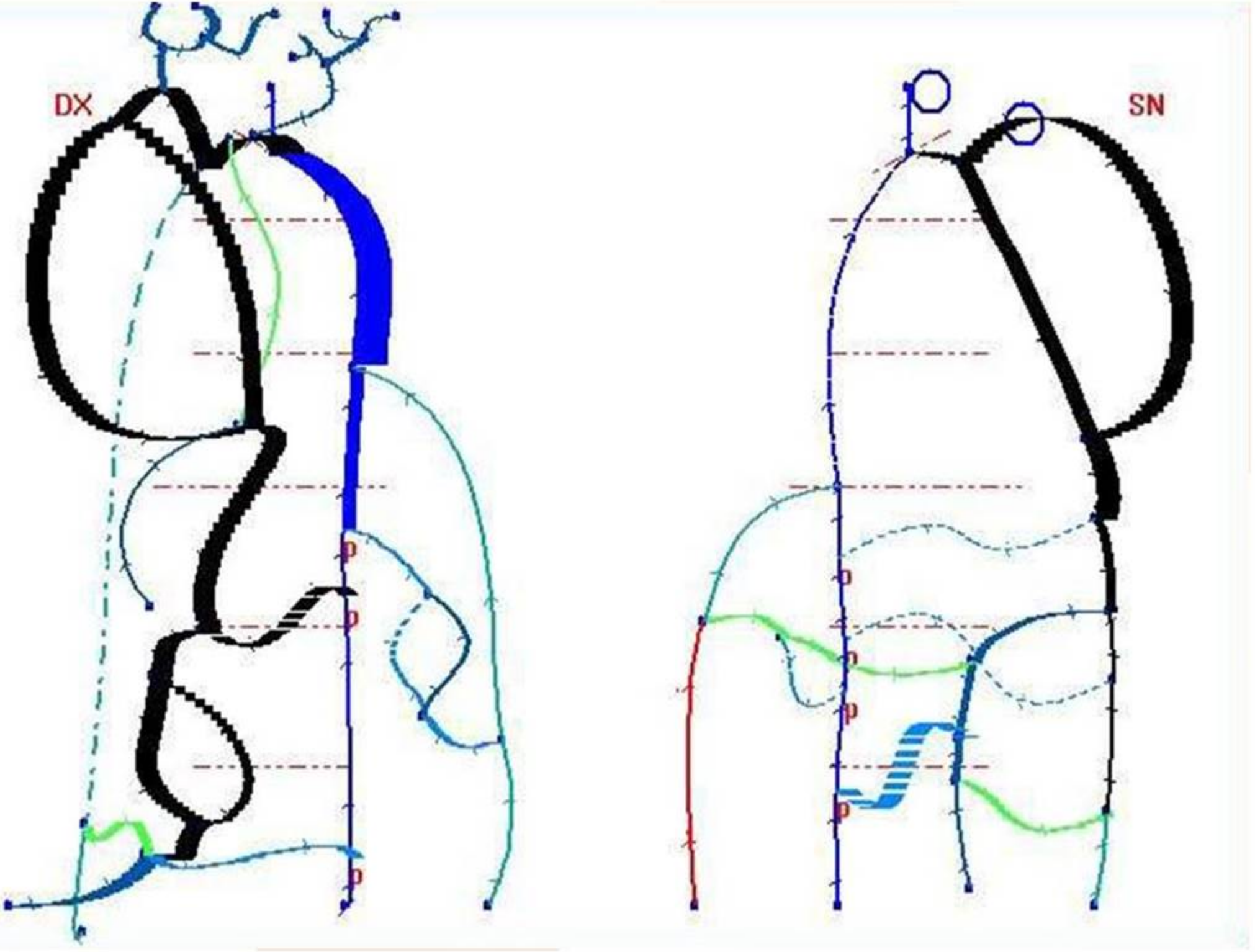
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The Journal of Theoretical and Applied Vascular Research (JTAVR) publishes scientific papers on vascular diseases, biological research, history and philosophy of science.

Manuscripts are expected to comply with the instructions to authors which conform to the Uniform Requirements for Manuscripts Submitted to Biomedical Editors by the International Committee of Medical Journal Editors (www.icmje.org/).

Submission of manuscripts

Papers should be submitted directly online to the Editorial Office at the Fondazione Vasculab ONLUS website: www.vasculab.eu/jtavr/submissions.htm

The journal adheres to the principles of the Helsinki Declaration (www.wma.net/en/30publications/10policies/b3/index.html) about research concerning human beings and to the International Guiding Principles for Biomedical Research Involving Animals (www.cioms.ch/publications/guidelines/1985_texts_of_guidelines.html) recommended by the WHO. In addition, the journal strongly supports alternative non-animal experiments, in order to Replace, Reduce and Refine (3Rs) animal experimental designs.

For complete information about publication terms please contact the Editorial Office of JTAVR, Fondazione Vasculab impresa sociale ONLUS, via Francesco Cilea 280 Italy - Phone +39-081-7144110 - E-mail: jtavr@vasculab.eu.

Open Access Publication

All manuscripts submitted to JTAVR are assumed to be submitted under the Open Access publishing model. In this publishing model, papers are peer-reviewed in the normal way under editorial control. Submission and reviewing process are not charged. When a paper is accepted for publication the author is issued with an invoice for payment of a publication processing fee (see www.vasculab.eu/jtavr.xml). Payment of this charge allows JTAVR to recover its editorial and production costs and create a pool of funds that can be used to provide fee waivers for selected authors, for instance for invited authors, authors of papers on history and philosophy of science and for authors from lesser developed countries (see below).

Free download

Published papers appear electronically and are freely available from our website. Authors may also use their published .pdf's for any non-commercial use on their personal or non-commercial institution's website.

Commercial use

No articles from the JTAVR website may be reproduced, in any media or format, or linked to for any commercial purpose (eg. product support, etc) without the prior written consent of JTAVR and payment to JTAVR of an appropriate fee.

Publication fees

Initial publications of JTAVR (up to presumably one year) are free of charge for invited Authors and for the members or supporters of the Vasculab Foundation. For the Vasculab Foundation membership see www.vasculab.eu.

Article types

Editorials, original articles, review articles, systematic reviews and meta-analyses, randomised controlled trials, research protocols, case reports, therapeutical notes, letters to the Editor, guidelines, special articles (like history and philosophy of science), invited sessions, reprints of historical papers of actual interest.

In order to submit an article online, follow the step by step instructions at www.vasculab.eu/jtavr/submissions.htm

Preparation of manuscripts

Footnotes or endnotes

JTAVR does not encourage the use of footnotes. Generally, they are not used in medical journals, but they are tolerated, especially in articles in the field of history and philosophy. Footnotes or endnotes must be quoted in low caps romans in rectangular brackets.

References

- Only cited references can be included in the bibliography. They must be numbered in Arabic numerals, in the exact sequence as they are firstly cited.

- Bibliographical entries in the text should be quoted using superscripted Arabic numerals.

- References must be set out in the standard format approved by the International Commitee of Medical Journal Editors (ICMJE), as described in the document Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals www.icmje.org/icmje-recommendations.pdf.

A simplified but comprehensive list is given in www.nlm.nih.gov/bsd/uniform_requirements.html.

Citation examples

Standard journal article

List the first six authors followed by et al.

Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002 Jul 25;347(4):284-7.

As an option, if a journal carries continuous pagination throughout a volume (as many medical journals do) the month and issue number may be omitted.

Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002;347:284-7.

Books and Monographs

Author(s) and editor(s)

Breedlove GK, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services; 2001.

Chapter in a book

Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113.

Electronic materials

Homepage/Web site

Cancer-Pain.org [Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

Vasculab mailing list

Provided you know the number '#' of the message, the format of the citation here follows, where the date of the last access is required (following the Vancouver style) and the symbol '#' must be replaced with the effective number of the message:

Author(s) name(s). Vasculab Yahoo Groups. The Vascular List. Message '#'. https://it.groups.yahoo.com/neo/groups/vasculab/conversations/messages/# Accessed on line on 'date of last access'. A (free) subscription to Vasculab is required.

Historical monographs

The format will be specified in next future.

File of tables

Each table should be submitted as a separate file. Formats accepted are .doc and .rtf. Each table must be numbered in Roman numerals and accompanied by the relevant title. Notes should be inserted at the foot of the table and not in the title. Tables should be referenced in the text sequentially.

File of figures

Each figure should be submitted as a separate file. Formats accepted: JPEG set at 300 dpi resolution preferred; other formats accepted are TIFF and PNG. Figures should be numbered in Arabic numerals and accompanied by the relevant title. Figures should be referenced in the text sequentially.

Histological photographs should always be accompanied by the magnification ratio and the staining method.

Color illustrations

Open Access papers appear electronically. As no printed issues of JTAVR are produced there are NO additional charges for color illustrations.

However consider that many people will print them in black and white. Thus for a better result in communicating your data, test also the black and white printing when choosing colors.

EDITORIAL

Aiming at a different View in Vascular Research

F Passariello*

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submitted: Jul 6, 2016, accepted: Jul 6, 2016, EPub Ahead of Print: Nov 30, 2016, published: Dec 31, 2016
Conflict of interest: None

Corresponding author: Dr. Fausto Passariello, afunzionale@tiscalinet.it

Fausto Passariello

Dear Readers and Researchers,

The Journal of Theoretical and Applied Vascular Research (JTAVR) aims at gathering contributes to vascular research, coming from biology, medicine and basic sciences like physics, fluid dynamics and bioengineering.

The journal is open also to the cultural aspects which lay behind our daily work, like models, epistemology, philosophy, history, in order to achieve a better comprehension of vascular phenomena.

As I often say, each patient who comes for a medical consultation deserves also a 3 minute, non-invasive and safe research work, privileging the cultural aspects and going far beyond our daily procedures, instead of stopping only at their material execution. Each patient indeed can teach us and being lazy in observing his/her data is an important loss and forever.

JTAVR has an online parallel voice in Vasculab, the Vascular List! (1990), which since 26 years is the most spread vascular list in the world. JTAVR is the Official Journal of the Vasculab Foundation ONLUS (2015), which aims to scientific research and to humanitarian missions. Vasculab, the Vasculab Foundation and JTAVR all three together share almost the same goals, of course with different realizations.

In addition, the journal strongly supports alternate non-animal models and any effort in order to Replace, Reduce and Refine (3Rs) the design of animal experiments.

The first issue of JTAVR is a practical example of this inter-disciplinary approach, covering almost all the topics cited above, as the Vasculab history and a general introduction to the 3Rs argument. Treating the theoretical issue of vascular branching, several points of view are placed together from history of science to mathematics, from theoretical biology to microscopic and 3D anatomical reconstructions.

Using a wider eye/chakra, JTAVR accepts papers which could never find a place together in medical journals. We in the Editorial Team of JTAVR hope that this inter-disciplinary environment will succeed in producing new interesting fruits in research.

Fausto Passariello

on behalf of the JTAVR Editorial Team.

The Vasculab Foundation history and mission

F Passariello*

*Fondazione Vasculab ONLUS, via Francesco Cilea 280 - 80127 Naples, Italy

presented to: the Hemodyn2015, Nov 2015, Naples, Italy
submitted: Dec 29, 2016, accepted: Dec 29, 2016, EPub Ahead of Print: Dec 30, 2016, published: Dec 31, 2016
Conflict of interest: None

Corresponding author: Dr. Fausto Passariello, afunzionale@tiscalinet.it

Abstract Vasculab, the Vasculab Fundation and JTAVR are 3 different entities, though they aim at similar goals. The Vasculab Foundation was born in July 2015, while Vasculab, the Vascular List!, is active since 1990, when it was a Fidonet Message List running on the Aquarius Bullettin Board System. Vasculab guests discussions about vascular diseases in an apparently free style. Discussions instead are moderated and driven gently towards scientific goals. The List is subjected to rules, which are summarized in policy documents. Vasculab serves as an Ideas Editor and as a shared basis to elaborate scientific contents, to organize conferences, courses and seminars. Nowadays, the Vasculab Online Events and Debates are considered important appointments for their educational value and research content.

Keywords Non-profit; Vasculab List; Internet; Vascular diseases; Philosophy of Science

Historical notes

The history of the Vasculab Mailing List dates long ago (26 years)[1]. At the origins in **1990**, Vasculab was a Message List, originated from the **AQUARIUS** Bulletin Board System **(BBS)** at the address **FIDONET 2:335/229**. The Fidonet Nodelist 042 for the Zone 2(Europe), Region 335(Italy), released on Feb 11th 1994, reported the Aquarius BBS as follows:
nodelist.042:,229,Aquarius,Napoli,Fausto_Passariello, 39-81-7144110,9600,V32B,V42B,XA
declaring a transfer rate of 9600 bps (really it was much higher) and the adoption of the V32B, V42B and XA protocols. The typical structure of **Fidonet**[2] was hierarchic, with an automatic scheduled exchange of information between computers in a peer-to-peer fashion. FidoNet was a world-wide organization and provided an effective but nowadays obsolete way to distribute information on a network[3].

In **1994** Vasculab was transferred on the Internet, stimulating people to write for scientific magazines and starting to organize scientific events. Today, Vasculab is a Yahoo! Mailing List, subjected to the general policy of the Yahoo! Groups.

The Vasculab Foundation

Recently a new chapter was added, as since July 2015 Vasculab is supported side by side by the Vasculab Foundation, a non-profit research organization[4]. One of the main goals of the Vasculab Foundation is to sustain the Scientific Research dedicated to Vascular diseases. In the background, but essential to scientific comprehension, there are the basic sciences, the neurosciences and biology in a wider sense, with an important extension to humanities, communication and divulgation. The JTAVR magazine[5] is another complex sustaining tool, which tries to achieve the same goals in common with the List and the Foundation. Aiming at solidarity too, the Foundation organized till now two phlebological missions toward disadvantaged communities, following a previous volunteer experience of almost 7 years. Vasculab strongly supports non-animal methods in medical experimentation, which is a difficult struggle and needs a long cultural and educational effort.

Vasculab management

Generally Vasculab guests news and discussions about very different topics. Discussions start spontaneously without any invitation, but they are maintained at the highest level, taking advantage of the presence of experienced people in the List. Reports, unusual topics and the collection of new ideas are only an example of the difficult work of Vasculab, which gathers all this material

together to serve as an ***Ideas Editor***. This feature of the List is a direct consequence of being a moderated list, where the content of the discussions is gently driven towards a scientific goal. Participation to Vasculab undergoes several ethical rules, with the essential requirement that people must respect each other: for instance, it is necessary to maintain a low tone in discussions. These requirements are well-known rules in the management of mailing lists and generally they are called **Netiquette** (Net-Etiquette) rules[6].

Vasculab has its policy, which dictates the behaviour for the correct management of the List. A special policy deals with the treatment of data, which must be anonymous and not related to recognizable patients.

The subscription and the active participation to the discussions automatically implies the **Vasculab Policy agreement** by the participant. Should there be any strong and unsolvable disagreement, participants should kindly consider to unsubscribe. **Advertising** of products and devices by companies is limited by several strict rules and generally separated from the scientific content of the messages. In addition, advertising is subjected to fees, in order to fund the activities of the List. On the contrary, scientific participation is free and not subjected to any fee, as it occurs in discussions between friends which meet together, coming from several parts of the world. Today, Vasculab gathers scientific updates, courses and meetings announcements together with scientific discussions in the field of peripheral vascular diseases. The Vasculab structure is typically non-hierarchic. Technically, it is a **moderated Mailing List**, with a fixed Moderator, owing to strong informatics requirements in the daily management. Moderating deals also with technical projects. Practically, discussions are **as free as possible**, with minimal changes in order to insure profitable discussions. Subscribers are stimulated to share information in a democratic environment. Interesting discussions and surveys are often summarized by selected participants for publication on international scientific magazines. In the last years a great amount on time was dedicated to the organization of the residential **Vasculab Conferences** and Seminars, which became an important appointment for researchers in the field of peripheral vascular diseases. Let me cite for instance the Hemodyn series, which is organized each 2 years in Napoli, as an update in peripheral hemodynamics. It is also a great honour that, during the first Hemodyn 2011, the President of the "Union Internationale de Phlebologie" (UIP), at that time Angelo Scuderi, was so stimulated by the scientific content, that he had the idea of organizing the Consensus of Hemodynamics[7], which was completed and later published in 2016, with the leading role of BB Lee. Thus, Vasculab succeeded again in its role of Ideas Editor! In addition, Vasculab always had and still has a good external relationship with the UIP, as well as with other scientific societies.

It is worth reminding the organization of the **Online Events** in the past years, which were specialized up-to-date discussions in the field of peripheral vascular diseases. An uncommon feature was the democratic election of the President at the start of the scientific event. I would just like to recall the Presidence of John Bergan in the Foam_PFO and the LASER_Floating Events.

During the last year, a new type of online activities, the Online Debates, was started as an evolution in the organization of the previous Online Events. Each Debate has one or more Discussants, together with several Panelists. Since Jan 2015, 4 Vasculab Debates, each 20-25 days length, were organized, with an undoubted success. JTAVR will summarize these Debates in next issues. As always occurs when an initiative or an institution has a long life, we all miss several eminent scientists. We gratefully remind their participation. Vasculab plans to organize the online memorial pages to honour their personal contributes.

Final remarks

Vasculab is not a remote continuous meeting. Essentially a meeting stimulates the discussion, but constrains it into the scientific content of the accepted presentations. The debate is then obliged to follow several pre-defined tracks.

In Vasculab indeed, **each participant can democratically become a protagonist**, thus practically inverting his/her academical role. This happens of course more often in Vasculab during a spontaneous discussion, but less during events and debates.

In addition, everybody, who had the unpleasant but common experience of not having enough time to explain his/her personal point of view, will favourably appreciate the possibility of writing without any limit, although respecting the rules. The online discussion is then a different scientific tool, compared to the Meeting. Let me just summarize several points:

- **No length limit** in questions and answers;

- **No immediate answers**, thus there is time to think (answers travel by mail and are moderated);

- The discussion resembles more a **dialogue between peers**, than a hierarchic dialogue;

- Meetings are conditioned by great organization expenses; there will never be a big meeting about a very rare disease or an orphan drug; online events instead allow discussions about **uncommon and non-remunerative topics**.

The long experience of Vasculab testifies a **continuous appreciation** of the participants. Vasculab thanks them all for their daily attention and availability to be part of the new continuously proposed initiatives.

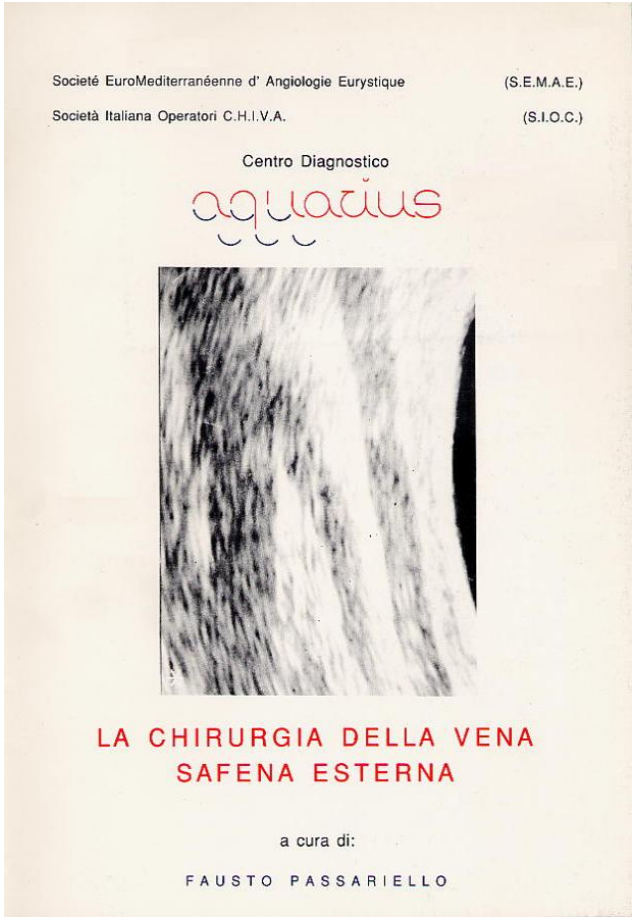

Figure 1 - Short saphenous vein surgery (1991).

Figure 2 - The well tempered encephalon (1997).

Figure 3 - The Sclerosing Foam and Patent Foramen Ovale (PFO) Online Event (2007).

Figure 4 - The Systodiastolic Online Event. The systodiastolic reflux in venous dynamical manoeuvres and its interpretation (2013).

Appendix I - Vasculab Activities

The Vasculab web site: http://www.vasculab.it

Books

La Chirurgia della Vena Safena Esterna. 1991[8]. (Figure 1)
L'encefalo ben temperato. 1997[9] (Figure 2).

Chivaref

Chiva references in the world[10]. Last updated in 2004.

The Hemodynamics Courses and the International School of Vascular Ultrasound

- MEVc (2008-2009), SHDyn (2010-2011), Ecocolordoppler, Emodinamica Venosa. (2014), ClidLy (2011).

The Vasculab Conferences

Short Saphenous Vein Surgery 1991, The well-tempered Encephalon 1997, Clinica e Diagnostica delle Linfopatie 2006, Lymphup 2011, Hemodyn 2011-13-15, Compression 2012, Venous Thromboembolism 2013-15, Bedside Oxygen Cascade 2014.

The Online Events

- The ICIVP Project: The Project on **I**nformed **C**onsent **I**n **V**ascular **P**rocedures. A distributed collective work[11].

- Foam_PFO: SCLEROSING FOAM AND PATENT FORAMEN OVALE. The on line discussion on the M.V. Forlee JVS paper. President: John Bergan[12 13] (Figure 3).

- CHIVALAB: THE CHIVA LABORATORY. CHIVA 20 YEARS LATER. President: Paolo Zamboni.

- Laser-Floating: Floating Thrombus and Laser Endovascular Therapy[14 15].

- PhysBiol: Physical and Biological Variables in venous diseases.

- WHAT-MD: The WHAT-MD Classification for Venous Interventions

- Systodiastolic: The systodiastolic reflux in venous dynamical manoeuvres and its interpretation (Figure 4).

The Vasculab Debates

VTE_Risk (Fedor Lurie), L-Phys (Waldemar Lech Olszewski), KTrenaunay (Byung Boong Lee), Biofilm (Carolina Weller, Giorgio Guarnera).

The Surveys

The Foam PFO Survey, The Chivalab Survey, The SVT Survey[16], Compression for venous Ulcers, Sugar-Honey[17], the CEAP Survey[18].

Outstanding discussions

Venous valvuloplasty[19].

Venous diagnostics

The Vasculab Manoeuvre[20].

Software

The VNet Model.

Appendix II - The Vasculab Identity Card

(at the date of Dec 30th, 2016)

Name: Vasculab (The Vascular List!)
Typology: Mailing List
#subscribers: 2345
Language: English (tolerated also Italian, French and Spanish)

Countries

Europe

Italy, Spain, Portugal, United Kingdom, Ireland, France, Belgium, Nederland, Denmark, Germany, Austria, Switzerland, Sweden, Monaco, Finland, Norway, Estonia, Poland, Chzech Republic, Slovakia, Russia, Slovenia, Croatia, Bosnia, Republic of Serbia, Bulgaria, Georgia, Ukraina, Hungary, Moldavia, Romania, Macedonia, Greece, Cyprus, Turkey.

Africa

Algeria, Morocco, Tunisia, Egypt, Senegal, Cameroon, South Africa.

North America

USA, Canada, Hawaii

Central and South America

Mexico, Cuba, Panama, Colombia, Paraguay, Argentina, Brazil, Peru, Ecuador, Venezuela, Chile.

Asia and Australia

Iran, Iraq, Israel, Palestine, Japan, South Korea, India, Nepal, Pakistan, Thailandia, Vietnam, Taiwan, China, Japan, Micronesia, Australia, New Zealand.

Description

Vascular Diseases Discussion List, Vascular Ultrasound Diagnosis, Vascular Biomechanics, Chiva Discussion List, (Chirurgie Hemodynamique de l'Insuffisance Veineuse en Ambulatoire), Haemodynamic Venous Map (MEV), Minimal MEV, V N e t, the Model of the Venous Circulation, Micro-Circulatory Disorders, Lymphatic Diseases, Lasers in Vascular Diseases, Foam and Sclerotherapy.

Appendix III - Rules

Vasculab Policy

1) VASCULAB is a restricted Yahoo! Message List, open only to members

2) Active participation to the List must be approved by the Moderator.

3) Accepted members, mainly people of several professions involved in the management of vascular diseases, must respect generic "Netiquette" rules (Net Etiquette), as also the specific rules and the discussion topics listed in the first message they receive from the List.

4) Topics are many, but they all are scientific ones.

5) Direct consultation with patients is not allowed.

6) Drugs or medical devices promotion is forbidden inside the messages (as also hidden in messages, e.g. mail address of companies and their web sites).

7) Outside of the messages and clearly separated from them, commercial information can be given, though generally subjected to a fee.

Please read and respect also the rules included in the **VASCULAB DATA OWNERSHIP POLICY**. If these rules do not comply with you, it's better to unsubscribe. Participating actively to the discussions automatically implies the Vasculab Policy agreement.

Vasculab Data Ownership Policy

1) Patients data

a. They have to be anonymous, in respect of the privacy, thus there must be no way to identify the patient. This is up to the physician who uploads data.

b. The physician must be the owner of clinical and imaging data or must declare who is the owner and how he got the authorization to publish. Use of unauthorized scientific materials owned by others is a fraud, though we have not nowadays a satisfying method to struggle against it. In conclusion, it is supposed that the uploader is also the owner of data or has the authorization to do it.

c. As an appendix to the previous point, data cannot be published if they are included in an ongoing judgement. Publication of data to gather opinions of Vasculab participants nor the citation of Vasculab discussions is permitted as an attached document to a still open legal report or trial. Participants are strongly requested to avoid these embarrassing situations.

2) Reuse of data

a. The uploader retains the ownership of his data, with the only exception that they can be used only for the official report of the discussion, only if organized by the Vasculab List and citing the owner. If the owner doesn't want to let these data officially reused for the report, he is strongly asked not to upload them. (However, only a limited amount of uploaded data will be reused. In addition, uploaded files are sent only once to the participants, but are not stored on the Yahoo! Website.)

b. People participating to the discussion must consider that data uploaded can be publicly available or instead only attached confidentially. According to this, reuse of data is generally not allowed, unless there is a confidential authorization of the owner.

Though Vasculab strongly defends the ownership and the copyright of data attached to its discussions, Vasculab cannot be considered responsible at all for the use of the uploaded data in a wrong or fraudulent way by third parties. Vasculab can testify the ownership of data or the one who first uploaded them, but cannot make more. For any legal issues and controversies the competent Forum is in Naples, Italy.

Acknowledgements

Vasculab experience started long ago and I would like to thank my past coworkers Amelia Morra, Tiziana Spiezia and Sabina Mazzarella, whilst since 1998 I shared the management of the Vasculab List with my collaborator Iolanda Palma.

References

1) Passariello F, Palma I. The Vasculab History. Presented to Compression 2012, a Vasculab Seminar, Sorrento (Napoli), 2012. Acta Phlebologica 2012 Agosto;13(2):109-11. Available at the address http://www.vasculab.it/compression2012/panel/sh/35TX_PassarielloF_The_Vasculab%20History.pdf at the date of Dec 30, 2016.

2) Bush R. FidoNet: Technology, Use, Tools, and History organization. Available at the address https://www.fidonet.org/inet92_Randy_Bush.txt at the date of Dec 30, 2016

3) Bush R. FTS-0001. A Basic FidoNet Technical Standard. Available at the address http://ftsc.org/docs/fts-0001.016 at the date of Dec 30, 2016.

4) Vasculab.eu [Internet]. Napoli: The Vasculab Foundation impresa sociale ONLUS. Available from http://www.vasculab.eu/ at the date of Dec 30, 2016.

5) Vasculab.eu [Internet]. Napoli: The Journal of Theoretical and Applied Vascular Research. Available from http://www.vasculab.eu/jtavr.xml at the date of Dec 30, 2016

6) Hambridge S. RFC1855 document, available at the address https://tools.ietf.org/html/rfc1855 at the date of Dec 30th, 2016

7) Lee BB et al. Venous hemodynamic changes in lower limb venous disease: the UIP consensus according to scientific evidence. International Angiology 2016;35(3):236-352.

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10) The Chiva references in the world web site. Available at the address http://web.tiscali.it/afunc/chivaref.htm at the date of Dec 30th, 2016.

11) The **I**nformed **C**onsent **I**n **V**ascular **P**rocedures. Available from http://web.tiscali.it/afunc/icivp/start.htm at the date of Dec 30, 2016.

12) Passariello F: Focus on an Internet Meeting. ACTA PHLEBOL 2007;8:63-5

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NON-ANIMAL NEWS NEWSLETTER

The importance of being the change

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submitted: Dec 23, 2016, accepted: Dec 23, 2016, EPub Ahead of Print: Dec 23, 2016, published: Dec 31, 2016
Conflict of interest: None

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Abstract A conference entitled *"Stop Vivisection Counter-Conference"* and focusing on the European Citizen Initiative (ECI) "Stop Vivisection" discussed the state of the art of non-animal alternatives, in terms of funding, legal aspects and official response of the EU Commission to the ECI, on December 6th 2016 at the EU Parliament in Brussels. The ECI by Stop Vivisection reached over a million certified signatures and stated: "Considering clear ethical objections to animal experiments and solid scientific principles that invalidate the "animal model" for predicting human response, we urge the European Commission to abrogate directive 2010/63/EU on the protection of animals used for scientific purposes and to present a new proposal that does away with animal experimentation and instead makes compulsory the use - in biomedical and toxicological research - of data directly relevant for the human species". A call for change demanding a clear and specific response by the European Commission to move to human-based research, as more specific and predictive for the human species. The results of the ECI and the response by the EC are discussed here, with particular focus to what prompts future changes and what it is expected by the Commission in response to the EU citizens.

Keywords Replacement; Alternatives; non-animal methods; non-animal approaches; Stop Vivisection; European Citizen Initiative; European Commission; ECI

On December 6th 2016 the *"Stop Vivisection Counter-Conference"*[1] expressed its view at the EU Parliament in Brussels, in a conference focusing on non-animal alternatives, the state of funding and the legal aspects of the official response of the EU Commission to the European Citizen Initiative (ECI) by Stop Vivisection. An ECI is an invitation to the European Commission to propose legislation of EU competence and needs to be supported by at least one million EU citizens, coming from at least 7 out of the 28 member states, this allows citizens to participate directly to changes to the EU law affecting all members states. Stop Vivisection is one of the three ECI to have been successfully completed to date, raising 1.173.130 certified signatures. As stated in the EU web site[2] this ECI had as main objectives: "Considering clear ethical objections to animal experiments and solid scientific principles that invalidate the "animal model" for predicting human response, we urge the European Commission to abrogate directive 2010/63/EU on the protection of animals used for scientific purposes and to present a new proposal that does away with animal experimentation and instead makes compulsory the use - in biomedical and toxicological research - of data directly relevant for the human species". A call for change urging a clear and specific response by the Commission to move to human-based research, as more specific and predictive for the human species.

Amongst its requests, the ECI asked the Commission to prompt an analysis of the state of the art of animal experiments, in terms of its putative effective value as predictor of human responses. In reply to the ECI the Commission organized a Conference aimed to connect and exchange information on animals' use and alternatives entitled *"Scientific Conference: Non-Animal Approaches - The Way Forward"*[3] the 6-7th December 2016. At the same time, the *"Stop Vivisection Counter-Conference"*

was occurring at the European Parliament, arguing that the Commission response was not sufficient, the time allocated to discuss on the actual usefulness of the animal model, at the Commission's Conference, was too limited and could not provide a constructive evaluation of the animal model, as requested by Stop Vivisection. Moreover, the time proposed to the Stop Vivisection promoters, in their opinion, was insufficient to illustrate their arguments and prompt real change. Above all, it was argued at the Counter Conference, that the Commission did not answer the specific questions and requests from the EU citizens via the ECI, but was avoiding answering in details to the scientific arguments and detailed evidence against the use of animals in science and research, reported by Stop Vivisection in the attached document, as a dossier provided to the Commission as supporting evidence[4] . The ECI also asked the Commission to stop animal experiments and revise the 63/2010/EU[5] , at least until properly evaluated using retrospective data analysis, both in toxicology and regulatory testing, as well as basic and applied research, as the animal model and animal experiments, they argued, have never been "validated" against their effective usefulness for humans. Using advanced human-based methods and technologies needs to be promoted, financed and pushed by legislation, financial commitment and exchanged knowledge. The *"Stop Vivisection Counter-Conference"* proposed, as well as confirming the urgent need to stop and take stock on animal experiments to date, to launch their "phasing out", as a social and institutional objective. The request to stop and take stock springs from the need to obtain a more efficient and safer research for humans and protect the human species, relying on human data and not extrapolating irrelevant data from animal models.

Amongst the speakers were Gianni Tamino, Andre Menache, Ray Greek, Michèle Rivasi, Elisabet Berggren and Candida Nastrucci. The main topics of the Counter-Conference, were concerning the lack of scientific validity of the animal model and animal experiments to predict a response in humans to drugs, therapies or human diseases, and the failure of the animal model in all areas of research. Personally, I concentrated my talk on the need to end the concept of 3Rs, to focus on the 1R of Replacement, that is the #final goal of full replacement# of the Directive 62/2010/EU. Furthermore, I reported the lack of funding for Replacement Alternatives and the difficulty in finding funds to create Courses on Replacement Alternatives and non-animal methods and the need for education and exchanging information on non-animal methods amongst scientists. Also, I have suggested to give priority to improve on the scientific culture to use and develop non-animal models and experiments, in all areas of research, particularly in basic and applied research. All the speakers agreed on the great need for increased funding for Replacement Alternatives, non-animal methods and for education on these methods and strategies in research, in order to prompt the change to a non-animal paradigm for research. Furthermore, the urgent need to make concrete changes to the legislative, cultural and financial framework, in order to move efficiently towards a research using advanced models based on the study of human beings, and the use of these non-animal methods, was agreed by the speakers. The need for better and specific human-based research is impellent, also by regulatory authorities, using a more efficient scientific validation procedure, as well as, using all the available replacement alternatives methods and strategies and the non-animal methods for research. Research, both basic and applied, and research & development, are the areas where the use of animals accounts for around 65% of all animals used in EU, although not compulsory by law.

Research is fundamentally important for health, environment protection and the maintenance of an healthy ecosystem. Therefore, science and institutions regulating research, should ensure that the best possible methods for research would be used and should evaluate all the results obtained and published to date, and attributed to animal experiments, before deciding if continuing to use animals, as the animal model was never "validated" against the scientific and predictive value for humans.

The *"Scientific Conference: Non-Animal Approaches - The Way Forward"*, organised by the Commission, reported some relevant non-animal research and projects, and included academia, industry and animal welfare societies. Amongst the speakers the most relevant talks concerning alternatives and non-animal methods were the presentation by Reyk Horland with the progress with the human organ-on-a-chip, and its possible use in the future to completely replace animals using only cells from human tissues to obtain specific answers, quantifiable and accurate. The talk by Jarrod Bailey related on an analysis of the use of animal models to predict human toxicity and drug safety. Thomas Korff spoke on the role of scientific journals to influence the implementation and use of alternatives for replacement in the scientific fields, in biology and medicine, and how they can be used to define how animal experiments are performed and how they can be evaluated. However, in my opinion, the most interesting and original part of the conference were its conclusions, in fact the final presentation summarized the combined updated comments, for every session of the conference, with the contribution by participants and external observers via the web. The combined comments helped to define the main bullet points concerning the "change" process, having as objective to "phase out# animal experiments. Amongst these points the most interesting to non-animal science were: "Citizens concerns should be considered"; "Funders, researchers and journals to come

together and create the right incentive". "There is significant concerns over how animal research is designed and how data is analysed"; "Some analysis show that experimental animals have no or very low predictive power for drug effect in humans". Some recommendations from Session 1 were: "Conduct a gap analysis of alternatives that are still lacking"; "Improve study design and data analysis (also for non-animal studies)"; "Invest higher proportion of funding in non-animal alternatives"; "Scientific peer review before starting the project"; "Need to publish negative results systematically - start with all publicly funded studies using animals"; "More "human" testing using cell based system or human on a chip"; "Funding should be dependent on quality of process, not just results". Session 2 stated amongst its conclusions: "Human cell based models and organs on a chip and its great potential, but still need an in vivo test to confirm if 3D - cell simulation reflects in vivo"; "Safety studies to investigate severe adverse effects could be replaced by in vitro models". Amongst the recommendations for session 2: "We need a coordinated research strategy/agenda", "Short term use more in silico productions and in vitro cell systems". "Long term human on a chip model of choice". "Development of patient DNA databases for research purposes". Session 3 conclusions added: "Need a paradigm change, moving away from 1:1 replacement of animal test with a non-animal one to integration of information and prediction of human effects". "International acceptance of alternatives is paramount"; "Need for political will". Session 3 recommendations included: "Need to carefully map uncertainties with animal tests"; "Need a new baseline and classification system to "validate" new methodologies"; Session 4 stated also: "New info not reaching approvers, funders company owners fast enough" "There is enough money to share knowledge, just needs to coordinate its use. Could the Commission help?". Some statements reported at the Conference, reflected the obsolete idea that the use of animals to model humans is still acceptable, as permitted, thereby the will to keep animals as models is reflected by comments, such as: "Human genomics helps to use animal models wisely and reduce use of larger species. Targeted gene editing of animals helps to exactly model a human disease". "Or Metabolism info and computer modelling can help bridge differences between species"; these statements demonstrated that the focus is still "adjusting" an animal to model a human being, and avoiding the real problem, that is that scientists using animals for experimental work are not studying the real human model, but they rely on animals as models for humans. However, animal models had never been scientifically validated as such, and they were never rigorously reviewed or retrospectively analysed, as requested instead by the validation process for replacement alternatives needing to be unreasonably compared to an animal model, although related to a real human cell/tissue/being. Amongst the concluding remarks is significant: "Attitude is important in education and training, with the right attitude you will find the right alternative" admitting that much of the effort to implement the #change# must be a responsibility of each individual scientist. Conclusions from even more sessions followed. Some of the suggestions and conclusions were innovative and useful, but considering the slow progress of Replacement and non-animal use in basic and applied science, in research projects and publications, in regulatory testing, and considering that the 3Rs is a 1959 concept, the progress is far too slow and in actual fact is not prompting an end to animal experiments and a "phasing out". In Europe the number of animals used for animal experiments, as reported by the EC in the Seventh Report, is around 11.5 million animals per year, and the numbers are believed to be higher now and to increase every year, with more genetically modified animals that are not even included in the statistics[6] .

In the initial few lines of its response to the ECI the EC states: "The EU shares the Citizens' Initiative's conviction that animal testing should be phased out. This is the ultimate goal of EU legislation". This is also the view of many scientists, which ever too often feel obliged to perform animal experiments for basic science to be able to publish their non-animal research, although often based on human cells or tissues. To words and intents towards real change, proper actions must follow: more funding, more advanced legislations, more controls on implementations and sanctions, more training on non-animal research and alternatives to replace animals, in all areas of science and research. A change of paradigm must occur, which seeks moving towards and reaching a better and more specific human-based research, which will never again need to use animals to compare and conclude for humans, as a human will never be the same as another animal.

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NON-ANIMAL EXPERIMENTS REVIEW

Alternative models and methods to animal experiments in vascular diseases

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presented to: the Hemodyn2015, Nov 2015, Naples, Italy
submitted: Nov 5, 2016, accepted: Nov 21, 2016, EPub Ahead of Print: Nov 30, 2016, published: Dec 31, 2016
Conflict of interest: None

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Abstract The Vasculab Foundation aims to find alternative models to animal experiments. Even thinking that animal experiments are useful, it is necessary to respect the 3Rs rule: Replacement, Reduction, Refinement If the 3Rs strategy would be used in the daily experimental work, a strong reduction in the use of animals in laboratories would be observed. Only a few examples are reported here, in particular those referring to the vascular contest, although the argument is much wider in other fields of biomedical research. Other fundamental issues regard the real innovation and the conflict of interest unacceptable in human trials but accepted in animal research. Finally, a good researcher should be the one who defends the animals used in the experiments, taking the part of the animals, instead of gaining from their use only in the name of an untouchable research interest.

Keywords Animal rights, non-animal experiments, the 3Rs argument, replacement, reduction, refinement

Introduction

One of the proposals of the Vasculab Foundation is to find alternative models to animal experiments and this is a proof of love and respect on the part of the Foundation towards animal life. However, these reasons could be considered not sufficient to all scientists to find alternative methods to animal experiments. The first thing we need to ask about is

"whom is this message addressed to?".

What do I want to speak about? Do I want to speak about ethical issues? Well, I am in favour of talking about ethical issues, but I will not do that; or do I want to speak of the usefulness/uselessness of animal experiments? I could do that, but I don't want to do it, because I want to talk of a completely different topic:

Even if you think that animal experiments are useful, you must respect the rules

This is a practical topic addressed to researchers, it is not a struggle nor an ideal crusade.

What are the rules?

There are a lot of declarations, institutional declarations[1], associations[2] and scientific societies like the European Society for Alternatives to Animal Testing (EUSAAT)[3], which are involved in explaining these rules.

Let me report just an example of experiments which do not follow the rules. Generally, many people are not aware that scientists used for a long time several useless animal toxicological tests to study cosmetic drugs and home care detergents, while they could have simply used practical non-animal tests that were already available. Underestimating these facts has no ethical justification at all.

But even if you are convinced that the animal experiments are absolutely useful, you must respect the **3Rs** argument: **Replacement, Reduction, Refinement**. (Figure 1) Then there are other fundamental questions … and last but not least several final remarks.

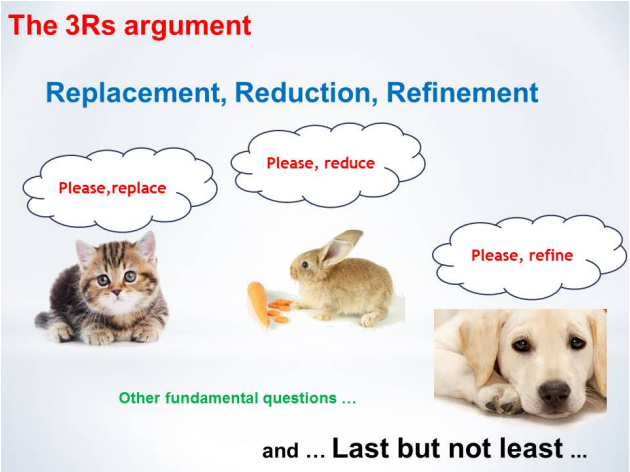


Figure 1 - The 3Rs argument

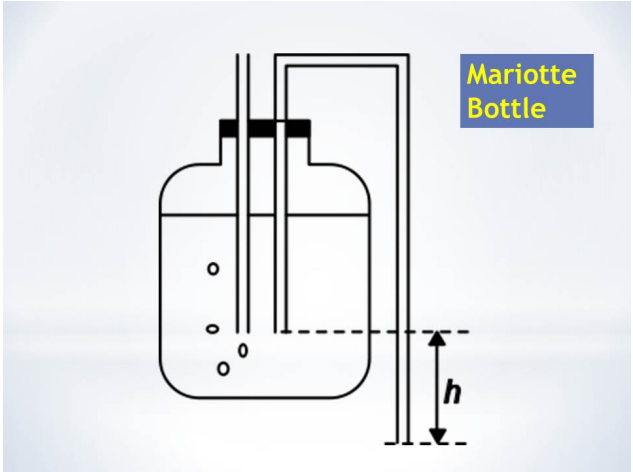


Figure 2 - Mariotte Bottle

Replacement

Please, **replace** *(if you can)*

your animal experiment with another (almost equivalent) experimental set, which does not require at all the use of any animal. As examples, we will illustrate only experiments of interest in vascular research.

Physical experimental sets

A classic replacement in hemodynamic measurements (pressure, flow, etc.) is provided by a Mariotte bottle[4] (Figure 2), used to fix the input pressure, and a Penrose drainage tube, which simulates a collapsible vessel, together with a hydraulic or an electrical manometer[5,6] (Figure 3). This experimental set is easily adapted to several research problems, involving also the effect of the tissue pressure on a collapsible vessel, easily simulated by a jacket with a second manometer. It is possible to change fluids input and calibres, resistance, height of the containers, and then take several measurements on flow, input and output pressures and so on, using a very low cost non-animal set.

The experiments can be repeated as many times as we want, provided we do not change the experimental set. For instance, injecting a glue inside the Penrose drain, alters the device and in order to set-up the subsequent experiment it is necessary to change the drain and any other altered component (Figure 3).

At the exit of the Mariotte bottle, the input pressure to the device is given by the difference in height between the level of the input tube in the bottle and the level of the experimental tube. As long as the liquid level is maintained higher than the extreme of the input tube, the input pressure remains constant.

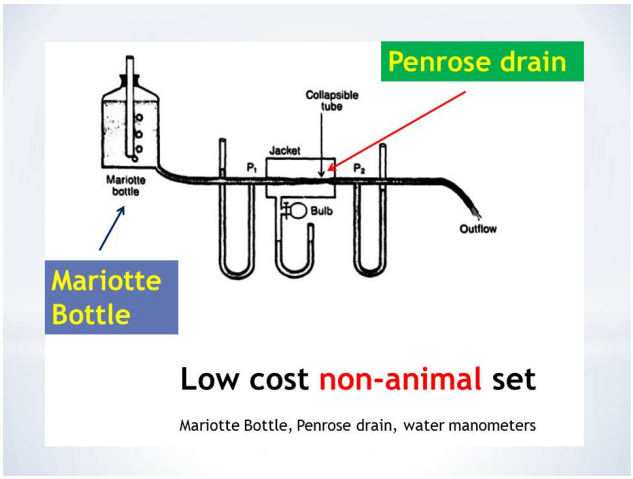


Figure 3 - Low cost non-animal set

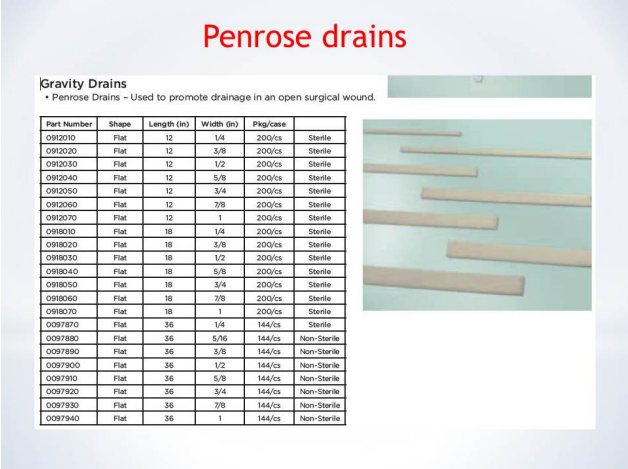
Penrose drains are generally used to promote drainage in open surgical wounds and are easily found on the market. They are available in flat shape and several sizes (12-36 in) and widths (1/4-1 in), as in sterile as well non-sterile packages (Figure 4).

Computer simulations

It is possible to estimate pressures and tensions (wall and venous valves) in a simplified clinical setting in the saphenous-femoral junction. Though the hidden complexity of computation, which however is faced only once and embedded in an opportune algorithm, measures are very simple and results can be very satisfying[7].

A lot of useful simulations can be organized, in order to understand better the experimental problem and to get help in designing the right experiment (see the "Refine" paragraph). Let me cite just one example constituted by

computer simulations made using Computational Fluid Dynamics programs[8], which can simulate very complicate models in hemodynamics.



Part Number	Shape	Length (in)	Width (in)	Pkg/case	
0912010	Flat	12	1/4	200/cs	Sterile
0912020	Flat	12	3/8	200/cs	Sterile
0912030	Flat	12	1/2	200/cs	Sterile
0912040	Flat	12	5/8	200/cs	Sterile
0912050	Flat	12	3/4	200/cs	Sterile
0912060	Flat	12	7/8	200/cs	Sterile
0912070	Flat	12	1	200/cs	Sterile
0918010	Flat	18	1/4	200/cs	Sterile
0918020	Flat	18	3/8	200/cs	Sterile
0918030	Flat	18	1/2	200/cs	Sterile
0918040	Flat	18	5/8	200/cs	Sterile
0918050	Flat	18	3/4	200/cs	Sterile
0918060	Flat	18	7/8	200/cs	Sterile
0918070	Flat	18	1	200/cs	Sterile
0097870	Flat	36	1/4	144/cs	Sterile
0097880	Flat	36	5/16	144/cs	Non-Sterile
0097890	Flat	36	3/8	144/cs	Non-Sterile
0097900	Flat	36	1/2	144/cs	Non-Sterile
0097910	Flat	36	5/8	144/cs	Non-Sterile
0097920	Flat	36	3/4	144/cs	Non-Sterile
0097930	Flat	36	7/8	144/cs	Non-Sterile
0097940	Flat	36	1	144/cs	Non-Sterile

Figure 4 - Penrose drains

Non-animal ultrasound pads and phantoms

Often a slice of turkey meat is used as a low cost ultrasound pad in emergency vascular access courses, while animal pads are not used for LASER endovascular treatments and venous sclerotherapy.

However, it is possible to build simply and quickly low cost pads for training in emergency and vascular access courses. Although the majority of these methods adopts homemade gelatine based ultrasound pads[9-11], it is possible to build homemade non-animal agarose ultrasound pads[12], including objects and Penrose drains to simulate tissue structures and vascular vessels for training in vascular access. Another alternative is given by high cost pads distributed by ultrasound companies, which offer a much greater reliability.

Replacing in favour of non-animal experiments can sometimes result on a reduction of costs and other times on an increase in costs, as these economic features depend mainly on the specific experimental set and design, thus a general answer cannot be provided.

Reduction

Please, **reduce** *(if you can)*

the number of animals required to perform your experiment. Benefit of statistics to compute the power of your experimental design[13].

What does it mean ?

Example of reduction

Given N the number of required measures to get the optimal power in your experimental design and M the number of the measures you planned, consider that:

- using **M** > **N** measures, you will waste animal life and/or provide useless animal pain.

- using **M** < **N**, again you will waste animal life and/or provide useless animal pain, because your experiment will have no power and no meaning at all and the sacrifice of all the animal used in the M measures will be absolutely vane.

- using **M** = **N** measures, or a little bit more (because some measures could be lost), you will do the right job, performing a reliable research with the right number of animals, therefore respecting the animal life.

Computing the right number N is not difficult, but requires a little knowledge of statistical methods[13]. This topic will be treated in depth later in a future article.

In addition, reducing has also an important effect in decreasing the overall cost of the experiment, decreasing the number of animals and the amount of materials used in each repetition, as well as the effort of people involved in the experimental work.

Refinement

Please, **refine** *(if you can)*

your experimental design and your underlying theory. Maybe, making computations and ameliorating your experimental planning, dedicating some effort to perfecting the design multiplying by 2 or 3 the time you already allotted to this job (NB! to be done only once), you will get a more meaningful experiment with better results, i.e. you will improve the experiment reliability.

Refining, can get a reduction in costs, when a simplification of the experimental set is achieved. Generally, a well done experiment can only get advantages in resources allocation from refining, while a badly conceived experiment can even require an increase in complexity and hence in costs. However, we should agree that a badly conceived experiment should never be carried on, being scientifically useless and in addition non-respectful of the animal rights and lives.

Really, the abundance of non-well-conducted animal experiments and the almost absence of systematic reviews and meta-analyses in animal research was recently pointed out in an important medical journal[14]. When translated later to human research these bias expose human participants to clinical trials to several harmful drug effects. Such a wastage of human and animal resources *"is as unethical in animal as in human research"*[14].

Finally, an important "refinement" regards the animal sacrifice after animal experiments. Do you really need this

sacrifice? For instance, you need to sacrifice an animal if you must get data from a lethal extensive tissue sampling.

On the contrary, could you help the animal in restoring its previous health conditions?

Other fundamental questions

Even if you will not communicate to other people the answers, nevertheless you have to stand in front at the mirror and ask yourself the following questions:

- Are you sure that you are making an innovative experiment which had not been already done before? Are you repeating instead an already published research? Repeating a research can still have a meaning, if you deliberately repeat a previous research of literature to check its reliability, but not if you are not aware of it in previous experiments. Knowledge is a prerequisite of good research, not being aware of literature is only ignorance.

- Which are the true motivations to perform your investigation? To which extent personal or team interests are involved ? In simple words, can you recognize in your experiment anything, maybe an economic involvement, which in human experiments could be easily called a "conflict of interest"? And, if this is the case, which justifications do you have to underestimate this conflict of interest while performing the animal experiment, while you feel obliged to take it into account while doing human research?

As an example, let's consider an experiment on the effect of a new drug, which is produced by a company. Having assets and personal interests in the company is generally considered a conflict of interest in human research, because an even unconscious bias, could be present in the results, vanishing then the usefulness of the human research. Why this identical argument does not stand for animal experiments? The same conflict would frustrate also the usefulness of the animal research.

The only explanation is that there is no Ethical Committee checking on the experimental design in almost the totality of animal research nor any Scientific Committee to evaluate the real usefulness of animal experiments.

Last but not least…

Consider that nothing (except some very weak laws) defends the animals.

As you are the planner and the owner of your experiment, you must take the part of the animals. Nowadays no one maybe will control you, but remind yourself that you are the only one who can defend the animals you want to use in your experiments, you are like a supervisor, you are the one who owns their lives.

Do not betray them!

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Wilhelm Roux (1850-1924) and blood vessel branching

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presented to: the Hemodyn2015, Nov 2015, Naples, Italy
submitted: Aug 28, 2016, accepted: Nov 8, 2016, EPub Ahead of Print: Dec 12, 2016, published: Dec 31, 2016
Conflict of interest: None

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Abstract The essay explores the work of the XIX century embryologist Wilhelm Roux (1850-1924) with particular focus on his research on the vascular system in the formation of his embryological theories.
The "Introduction" outlines an epistemological analysis regarding two of Roux's early works: his doctoral dissertation on blood vessel branching (1878) and his theoretical volume on functional adaptation (1881).
Section I, "Wilhelm Roux (1850-1924) and the prelude to *Entwicklungsmechanik*", delineates the epistemological background to Roux's academic formation: Darwinian revolution and biophysical research in physiology respectively shape the debates of the time that opposed historical to proximate causality and vitalistic causality to a physical-chemical one.
Section II, "Order in blood vessel branching: Roux's anatomical observations", introduces Roux's vascular observations: the identification of a regularity in vessel bifurcating angles and the verification that the whole vascular structure can be interpreted by the optimality principle of minimal physiological work.
Section III "Functional adaptation and blood vessel branching", introduces Roux's embryological concept of functional adaptation, which he intended to be a transposition of the Darwinian logic of variation and selection within the organism, and shows how Roux attempts to use this new concept to explain the developmental constraints on blood vessel direction.
Section IV, "A resourceful mechanistic explanation", stresses the epistemological ambiguity of the key concept of "mechanistic" causality and notes how this prevents giving a perfectly coherent picture of Roux's thought on development in the two texts examined.

Keywords Wilhelm Roux; Vascular System; Experimental Embryology; Hemodynamics; Functional adaptation; Internal selection

Introduction

This essay explores the work of the XIX century scientist Wilhelm Roux with particular emphasis on the role played by the vascular system in the origin of his embryological theories.

In the late XIX century, physiology and comparative anatomy had already inspected the structure and the functioning of the vascular system in order to understand metabolism (C. F.W. Ludwig 1816-1895) and pathology (J. F. Cohnheim 1839-1884), and to find out taxonomical proximities between species (E. Geoffroy Saint Hilaire 1772-1844).

In this context Roux's questions concerning the developmental appearance of vascular structure were a complete novelty as were his attempts to get a deeper look into the theoretical principles which are used to explain the details of blood vessel branching.

Of Roux's explanatory principles, we separate his appeal to hemodynamical laws from his use of the concept of functional adaptation.

The theory of development by functional adaptation is extensively addressed in the work "Der Kampf der Theile im Organismus" (1881) which will be quoted here in the French translation "La lutte des parties dans l'organisme" (2013)[7] .

While the aim of Roux's work is highly theoretical, his writing is profusely annotated with empirical details about experiments and scientific observations, blood vessel branching playing the role of a paradigmatic case study.

Roux's interest in vascular morphogenesis had already started during his university period spent at the Jena Medicine Faculty (1873-1877) where, in 1877, he defended his doctoral thesis about liver blood vessel branching, published in 1878 under the title "*Ueber die Verzweigungen der Blutgefasse des Menchen*[8] " (tr. eng. "On the bifurcations of human blood vessels").

We know from this and further publications (Roux 1879)[9] that he personally performed most of the experiments he describes, thus assuring both observational amplitude and reliability of his conclusion.

However, the repeated appeal to vascular analyses in Roux's early research, firstly in his doctoral thesis (1878) and later in the nearly "philosophical" work on functional adaptation (1881), does not point to a common theoretical framework. Indeed different questions underlie the two texts:

- the former (1878) is mainly concerned with the relationship between form and function in the vascular architecture and inspects the vascular system with the clear aim of finding anatomical generalizations that correspond to an optimal physiological condition,

- the latter focuses on the embryological causes of vascular morphology, with particular emphasis on the explanation of morphological development traits that show a kind of adaptation to induced stimuli. In this latter case, the focus is on the relationship between form and function but in a clearly different sense: functionality is the capacity of a developing organism to react to internal-external stimuli and arrange its form accordingly, that is, in order to cope with them.

There are certainly some points of intersections between the two texts and their related questions: in his doctoral dissertation, apart from relating the vascular architecture to a principle of minimum physiological work, Roux also addressed the issue of embryological causes, though he was said to have done it naively (Kurz et al. 1997[10]). This morphogenetic interest, described in the section III.1 of his thesis ("Gestaltende Wirkungen der hydraulischen Kräfte in Röhren bewegter Flüssigkeit") places this early text in closer contact with his following research on developmental causes.

As Churchill (*Dictionary of Scientific Biography*[11]) noted, "even at this early stage in his career Roux did not confine his generalizations to a descriptive equation. […] By making an analogy between hydrodynamics and hemodynamics, Roux implied a search for a causal connection between function and form" (p. 571). Otherwise said, as early as 1878 Roux was looking for mechanisms underlying vascular formation[i] [12] . We will see that the term "mechanism" and the correlated notion of "mechanistic explanation" have a different meaning in the two texts (1878, 1881): a physical (hemodynamical) force in the former and a statistical (population) process in the latter.

In order to understand the difference between the two senses of "mechanistic explanation" employed in the two texts, we need to put Roux's embryological theory into what we call the appropriate epistemological context. In this essay, the term "epistemology" will be used to refer to the overall set of scientific (and sometimes extra-scientific) assumptions constraining and defining the space of possible valid questions to be posed in a certain discipline (Rheinberger 2010[13]).

From this epistemological point of view, if we really want to understand what "explaining" means when it is used either with respect to the hemodynamical laws or to the concept of functional adaptation, we need to frame these terms in the broader history of their discipline.

Both those concepts fall within the field of Experimental Embryology, which is the English translation for *Entwicklungsmechanik* (literally, developmental mechanics). More accurately, the foundation of *Entwicklungsmechanik* dates back to the publication of Roux's *Einleitung* in 1895[14] , both a manifesto for a nascent discipline and an introduction for the new periodical Archiv fur Entiwicklungsmechanik (1895-1924). Roux's doctoral thesis on vessel branching (1878) and his work on functional adaptation Der Kampf der Theile im Organismus (1881) precede the 1895 manifesto. Nonetheless, the scientific questions at the basis of those works and their respective answers, that is the concepts of physical constraints and functional adaptation, obey the main tenets of *Entwicklungsmechanik* and can thus be considered a prelude to its future framing.

Wilhelm Roux (1850-1924) and the prelude to Entwicklungsmechanik

Wilhelm Roux (1850-1924) lived during one of the most exciting period for his discipline: at the end of the XIX century, biology metabolized the Darwinian revolution, was experiencing a second experimental turn through the rise of embryology and was approaching, through the rise of genetics, the discovery of quantitative tools able to describe the regularities of heredity. Progress in the study of evolution, development and heredity was setting the stage for biology to become the queen of XX century sciences.

Wilhelm Roux, whose French surname recalls his belonging to a Huguenot family dating back to the XVIII century, was born in Jena in 1850 and there, after joining up the army during the 1870-1871 French Prussian War, he started his studies at the Medical Faculty. At the time, the study of medicine embraced broader phenomena than human physiology and pathology and Roux was soon

fascinated by the lessons of the zoologist and anatomist Carl Gegenbaur, of his successor at the chair of anatomy, Gustav A. Schwalbe, of the physiologist Wilhelm Prayer and the Darwinian zoologist Ernst Haeckel (Churchill 1973[15]).

Haeckel in particular left a strong mark on Roux's further reflections. In the aftermath of Darwin's publication of the Origin of Species, Ernst Haeckel was one of the main supporters of the Darwinian revolution that explained the diversity of species as the result of evolution from a common ancestor through a continuous process of change crossing geological times with patterns (especially morphological ones) that could be explained through the aid of random variation and natural selection.

One immediate effect of Darwin's evolutionary theory was on taxonomy or the science of classification. From the turn of the XVIII century, taxonomy classified living beings according to their similarities and dissimilarities. There were functional classifications such as the one proposed by the French scientist George Cuvier according to whom functional similarities among organs (analogies) were induced by common conditions of existence and were a reliable empirical basis for classification. Others, such as the anatomist and embryologist Etienne Geoffroy Saint-Hilaire built their taxa by comparing the anatomical structure of species, that is, by comparing the relative disposition of their internal (bony) parts (Russell 1982).

Unlike comparative anatomy, Darwin interpreted homologies among species as a tool for reconstructing the timing of their evolutionary divergence from a single common ancestor and used tree-branching as the most convincing representation of life's diversity: in a tree-like representation, species similarities were converted into species proximity and the flat classification of comparative anatomy was substituted with a time dependent phylogenetic one.

When Roux attended Haeckel's lessons in Jena he could appreciate one of the main source of phylogenetic classification. Not only did Haeckel suggest, following his predecessor Karl Ernst von Baer's interpretation that in order to build faithful phylogenetic trees embryological characters had to be taken into account together with anatomical ones. He went further in saying that evolution proceeds in every species by "recapitulating", during ontogeny, all the life cycle of its immediate ancestor and by adding one "terminal" character to it. Within the Darwinian framework these processes of "recapitulation" and "terminal addition" can be understood as two mechanisms respectively explaining "heredity" and "variation" (Gould, 1977[16]).

From the point of view of Haeckel's theory of "Ontogeny" that "recapitulates phylogeny", no kind of explanation was needed in order to understand the specific succession of developmental stages in an organism's life cycle other than the past phylogenetic history of its species. He assumed that every organism, during its development, goes through all the phylogenetic steps which link the single common ancestor to its species-specific type. This meant that individual ontogeny was represented, and most importantly explained as a sort of rapid accumulation of phylogenetic steps.

This kind of developmental explanation is referred to as "historical" because it considers the causality underlying the succession of developmental stages in a single organism to be its past phylogenetic history, as if crystallized in heredity. No proximate embryological causes, amenable to experimental testing, are mentioned but only ultimate (phylogenetically based) descriptive ones.

It is exactly against this historical and descriptive explanation of ontogeny, represented by Haeckel's "biogenetic law" (Haeckel 1866[17]), that Roux developed his *Entwicklungsmechanik* (mechanics of development) where the term "mechanics" argues in favor of a step-by-step analytical (vs historical) and experimental (vs descriptive) analysis of developmental events.

Entwicklungsmechanik is thus primarily concerned with an emancipation of developmental enquiry from the yoke of phylogenetic necessity and provides a clear and fruitful break between evolutionary and developmental explanations (Allen 1975[18] , 2005[19]).

While Darwinism can help understanding the mechanisms accounting for biological diversity, developmental biology aims at discovering the immediate mechanisms accounting for embryological phenomena.

Therefore, Roux's interest in the law governing the formation process of the vascular system represented a break from the dominant Darwinian tradition: embryology started looking back to immediate causes of phenomena instead of using a historical (phylogenetic) notion of explanation.

However, how were those immediate causes to be conceived? At this point of the story, Experimental Embryology crosses the boundaries of another well-rooted epistemological debate. Not only do the characteristics of living beings have to be explained by making reference to immediate (and possibly experimentally corroborated) mechanisms but those mechanisms also have to be either distinguished from or eventually reduced to ones operating in non-living beings, in particular physical-chemical phenomena.

The mechanistic-vitalistic debate, between those who supported the existence of specific biological causality

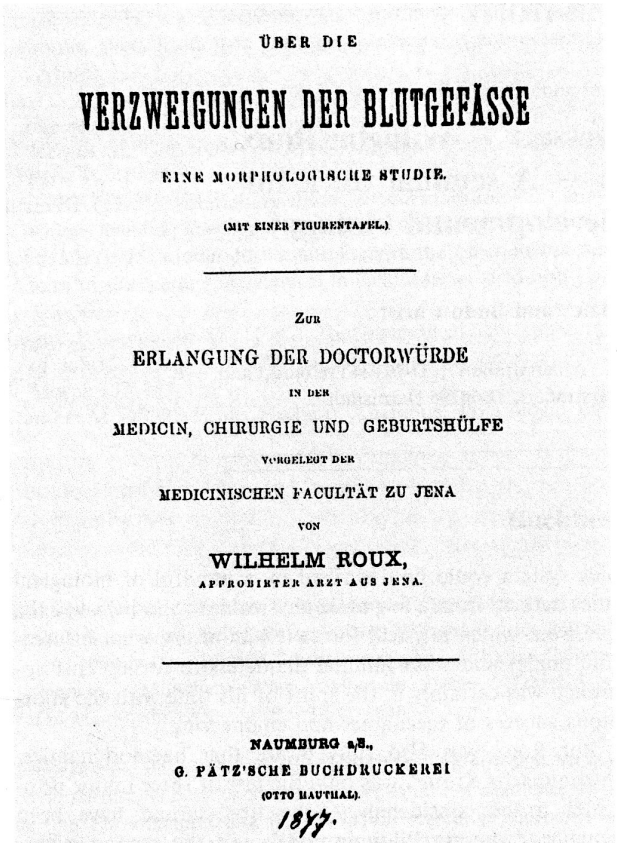
and those who equated it to physical-chemical principles, had a huge echo during the late XIX (C. Bernard, C. Ludwig, H. von Helmhotz) and early XX century (W. Roux, H. Driesch, J. Needham, J. Woodger) and Roux's epistemological concept of what must be considered as a scientific explanation for developmental phenomena lies fully within this important debate.

In *Einleitung*, Roux defines the new discipline as "the doctrine of the causes of organic forms" (Roux, 1895, p. 149) where the term "cause" has two different shades of meaning. Stricto sensu "in accordance with Spinoza's and Kant's definition of mechanism, every phenomenon underlying causality is designated as a mechanical phenomenon" (p. 150) where the term "mechanical" makes reference to the explanation of the phenomenon in terms of "movements of masses". Mechanical explanation is thus the only kind of explanation that grasps causality and in so doing constitutes an exact science. Accordingly, since the ultimate aim of physics and chemistry is to reduce "magnetic, electric, optical and chemical phenomena to movements of parts", the ultimate aim of embryology is to formulate its own explanation in mechanical terms, that is, to become "developmental mechanics". Besides this narrow sense of causality, which restricts scientific explanation to purely mechanical causes[ii] , there is a second carefully crafted path to formulate a causal explanation in embryology: lato sensu, a "causal explanation will always consist in tracing back a particular phenomenon to modi operandi of more general validity", that is, to (-non-mechanical- physical, chemical or biological) reliable generalizations, constantly valid under the same set of conditions[iii] [20] . According to this broader sense, causality is not restricted to mechanistic (that is mechanical) explanation and, though the ultimate aim of developmental mechanics is to attain such physical-chemical reducibility of embryological phenomena, in those early days of embryology it was more productive to engage in a higher level biological explanation. For this reason, Roux made a distinction between causal statements explaining embryological phenomena referring to constant relationships between "complex", that is, non-reducible, components, and causal statements ultimately formulated in terms of mechanistic relationships between "simple (physical-chemical) components".

As Roux himself wrote "the too simple mechanistic conception on the one hand and the metaphysical conception on the other, represent the Scylla and Charybdis, between which to sail is indeed difficult, and so far by few satisfactorily accomplished" (Roux, 1895). Concretely, embryologists should always aim at mechanistic generalizations but the empirical impossibility of attaining this deeper level of explanation should not lead them to metaphysical generalizations about immaterial, causal forces or entities.

This internal polarity between mechanistic and non-mechanistic causality within Developmental Mechanics does not reduce the discipline's radical novelty with respect to the former descriptive and evolutionary approach to development that was dominant in the second half of XIX century embryology (Allen 2007[21]). Independently from whether it is a thoroughly mechanistic science or just a causal, yet not mechanistic one, Roux's *Entwicklungsmechanik* aimed at giving a proximate explanation instead of an historical one and, most of all, tackled the issue of ontogeny through experimental methods and techniques and not through comparative analysis of development in phylogenetically related taxa.

The next paragraphs will introduce Roux's vascular observations and try to frame the tone of his arguments concerning the vascular system within the confines of the first historical-mechanistic (proximate) and the other mechanistic (physical-chemical)-vitalistic debate.



ÜBER DIE

VERZWEIGUNGEN DER BLUTGEFÄSSE

EINE MORPHOLOGISCHE STUDIE.

(MIT EINER FIGURENTAFEL.)

Zur

ERLANGUNG DER DOCTORWÜRDE

IN DER

MEDICIN, CHIRURGIE UND GEBURTSHÜLFE

VORGELEGT DER

MEDICINISCHEN FACULTÄT ZU JENA

VON

WILHELM ROUX,

APPROBIRTER ARZT AUS JENA.

NAUMBURG a/S.,

G. PÄTZ'SCHE BUCHDRUCKEREI

(OTTO HAUTHAL).

1878.

Figure 1 - Wilhelm Roux's PhD dissertation on blood vessel branching (Roux 1878). Title page.

Order in blood vessel branching: Roux's anatomical observations

"*Già da studente, preparando la sua dissertazione di dottorato, egli ricercava le cause dei fatti esaminati,*

cioè della forma del lume delle diramazioni vasali, tema assegnatogli dallo Schwalbe, continuatore di Gegenbaur. Egli riconobbe così che i vasi sanguigni si diramano secondo le leggi idrodinamiche, adattandosi cioè da una parte alla forza idraulica della corrente, dall'altra agendovi lo stimolo ad accrescersi ed insieme la inibizione ad accrescersi della tunica interna". Così egli intravedeva quella che chiamò la "lotta tra le parti degli organi", e per la prima volta dimostrava come lo sviluppo e l'accrescimento di un organo influiscono in un determinato modo su un altro organo."

(Castaldi, 1925, p.98[22])

Roux's empirical observations on the branched structure of the vascular system are extensively presented in his doctoral thesis "Ueber die Verzweigungen der Blutgefasse des Menchen" (1878, Figure 1) and further enriched in his "Ueber die Bedeutung der Ablenkung des Arterienstammes bei der Astabgabe" (1879). At the time those texts were written, Roux was ending his formal education in Jena (1878) and had just enrolled at the Hygienic Institute in Leipzig to do laboratory analysis. His work was strongly influenced by Schwalbe's interdisciplinary concern for the relationship between anatomical form and physiological function[iv] .

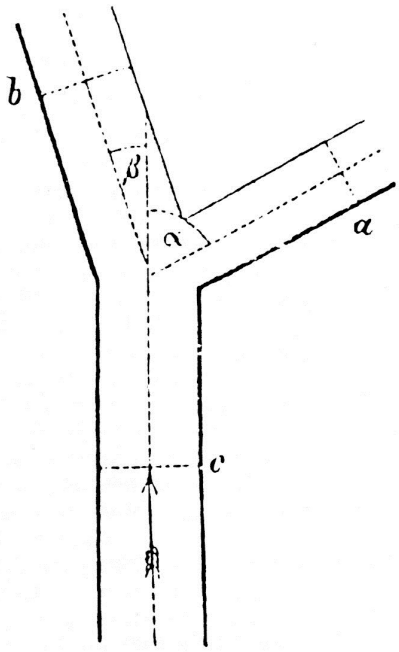


Figure 2 - Schema of an idealized vascular bifurcation (Kurz et al. 1997). For explanations of symbols, see the text.

Central to Roux's doctoral dissertation is the observation of the structure of blood vessels in the human liver and an attempt to correlate it to some laws for regularities.

In order to make the vascular architecture visible, Roux developed an advanced version of the ancient technique of wax injection, which consisted of "injecting wax into the vessels and, upon dissolving the surrounding tissues" being "left with only a naked casting of the branches" (Churchill 1973, 570)[v] [23] .

He focused on two parameters, the lumen of the vessel and its bifurcating angle and managed to find a set of structural regularities, which gained a special, functional meaning if linked to hemodynamical laws.

In other words, the regularities of vascular system's structure showed a sort of optimality when analyzed through the lens of metabolic and embryological functioning.

If we consider an idealized bifurcation of a stem trunk into two branches, such as the one depicted in Figure 2, the diameters a, b and c represent the lumen of the respective vessels while the angles α and β represent the distance of the vessels from their stemming trunk, or their bifurcation angles.

A first observation emerging from Roux's work concerns the correlation between the lumen of the branches and their bifurcation angles, this correlation being the object of Roux's laws.

He isolates three kinds of possible bifurcations which differ with respect to the ratio a/b, a and b being respectively the diameters of the branch vessels (Figure 3)

In the first typology of bifurcation a/b=1, the bifurcating angles of the branches a and b from the main trunk c are equal (Figure 3a).

Differently, if the ratio a/b>1, the larger branch a will deviate from the main trunk of a angle α smaller than the bifurcating angle β of the smaller branch (Figure 3b).

Finally for a/b>>1 , the smaller branch b being tiny, has a bifurcating angle β between 70° and 90° (Figure 3c).

In a quotation of D'Arcy W. Thompson's "On growth and form", Roux's laws are generalized as follows:

- "if an artery bifurcate into two equal branches, these branches come off at equal angles to the main stem;

- if one of the two branches be smaller than the other, then the main branch, or continuation of the original artery, makes with the latter a smaller angle than does the smaller 'lateral' branch;

- all branches which are so small that they scarcely seem to weaken or diminish the main stem come off from it at a large angle from about 70° to 90°."

(D'Arcy W. Thompson, 1917, pp. 667-668[24])

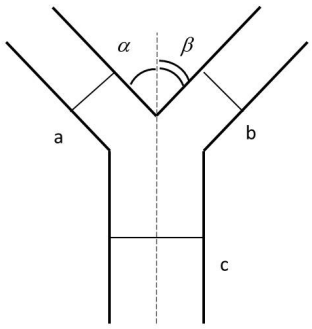


Figure 3a

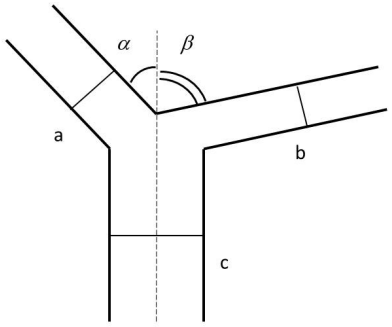


Figure 3b

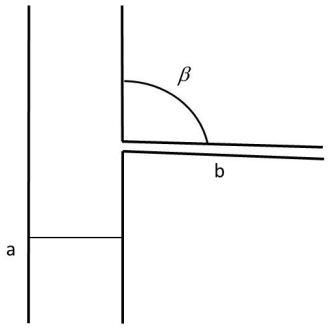


Figure 3c - Visual representation of Roux's laws on bifurcating angles.

Roux abstracted his laws from repeated empirical measurements, details of which are reported in his doctoral thesis (Table 1).

He used to take scrupulous note of the absolute values of the bifurcating angles and of the ratio a/b of the diameters of the two branches and his aim was to give a mathematical form to those correlations despite the numerous exceptions (Kurz et al. 1997).

However, what is constitutive about Roux's observations is the fact that the architecture of the branching pattern - at least with respect to the link between the ratio a/b and the bifurcating angles - could be explained by appealing to the constraints of hydrodynamic forces, that is, once we know the ratio a/b we can derive the bifurcating angles by applying to hemodynamical laws.

And we can do this precisely if we introduce a functional physiological criterion for minimum work: the whole branching structure of blood vessels is such as to accomplish circulation by exspending a minimum of energy. Given this physiological principle for optimality, the vascular system develops towards an optimal adult structure, this optimality being a sort of physiological end condition and not a properly morphogenetic one: what is physiologically optimal is the accomplished branched structure not the branching process.

Hemodynamical laws are a theoretical instrument for calculating (and eventually predicting) the relationships between the size of the branches and their bifurcating angles according to an optimality principle. They play the role of physical constraints on the (physiological) functionality of the system.

D'Arcy Thompson (1917) provides a clear explanation of this constraining role by underlining that energy loss is dependent on distance and lumen.

In Figure 4 the distance is the one between either C or D, the bifurcating points on the main trunk, and P, the external point to be reached by the vascular structure.

"If the large artery, AB, give off a comparatively narrow branch leading to P (such as CP or DP), the route ACP is evidently shorter than ADP but on the other hand, by the latter path, the blood has tarried longer in the wide vessel AB, and has had a shorter course in the narrow branch. The relative advantage of the two paths will depend on the loss of energy in the portion CD, as compared with that in the alternative portion CD', the latter being short and narrow, the former long and wide" (D'Arcy Thompson, 1917, p. 667).

Tabelle I.

α	a/b	β	α	a/b	β
18	0,83	14	55	0,33	7
18	0,88	17	54	0,61	10
			55	0,83	17
23	0,43	8			
23	0,72	14	60	0,45	15
23	0,80	18	60	0,55	27
22	0,88	19	57	0,87	39
22	0,95	21			
			66	0,75	27
24	0,66	11	68	0,83	48
24	0,85	18			
			83	0,38	4
25	0,57	16	86	0,44	9
25	0,77	18	76	0,86	32
27	0,71	11	Vereinzelt:		
27	0,91	20	90	0,43	34
27	0,91	21			
			102	0,43	4
36	0,40	15			
37	0,71	22	Ausnahmen:		
39	0,85	30	26	0,71	6
			46	0,83	6
40	0,68	9	48	0,91	6
40	0,83	25	60	0,50	12
42	0,81	28	17	0,83	19
42	0,83	32	44	0,74	0
			15	0,80	28
43	0,77	29	23	0,98	14
43	0,81	33	16	0,83	20
			27	0,87	33
49	0,71	10	20	0,83	33
48	0,88	11	16	0,94	23
			62	0,91	39
46	0,69	14	67	1,00	30
44	0,88	22	76	1,00	18
			32	0,87	8
50	0,58	17	15	0,73	13
51	0,86	29	27	0,91	36

Tabelle II.

α	a/b	β	α	a/b	β
38	0,23	16	Vereinzelt:		
38	0,76	27	29	0,45	18
			32	0,54	24
45	0,37	14	35	0,47	28
45	0,66	19	40	0,39	25
			Ausnahmen:		
45	0,43	21	49	0,43	22
46	0,59	35	53	0,59	13
46	0,87	37	54	0,51	19
			62	0,34	6
50	0,18	6	62	0,34	8
49	0,90	23	62	0,26	15
			62	0,23	16
51	0,37	6	63	0,45	16
51	0,41	8	64	0,47	20
			65	0,43	45
60	0,26	19	66	0,50	32
60	0,41	37	74	0,37	11
			75	0,29	10
63	0,25	16			
64	0,31	20			
66	0,47	32			
70	0,33	8			
71	0,66	22			
75	0,31	10			
74	0,33	14			
77	0,20	20			
78	0,38	25			
76	0,83	30			
80	0,50	18			
81	0,52	20			
80	0,59	27			
80	0,77	39			
88	0,36	20			
82	0,50	42			

Table I - Absolute values of bifurcating angles, α and β, together with the ratio of the branch diameters a/b in the vascular architecture of human liver.

Significantly, at the end of this quotation D'Arcy Thompson introduces Roux's laws.

As previously stated, the hemodynamical constraints are only related to the bifurcating angles of the branches while both their respective diameters and their destination (in Figure 4 the point P), at least in this first formulation, are considered as independent parameters of the system.

But, if the bifurcation angles of the branches can be calculated from their diameters, how can those diameters be calculated with respect to the size of the stem trunk?

Roux never explicitly addressed this issue but there is evidence in his doctoral thesis that he knew that his empirical observations on the relationships between branch and trunk diameters could be brought back to the same principle of optimality which had already proved fruitful in detecting the bifurcation order (Kurz et al. 1997).

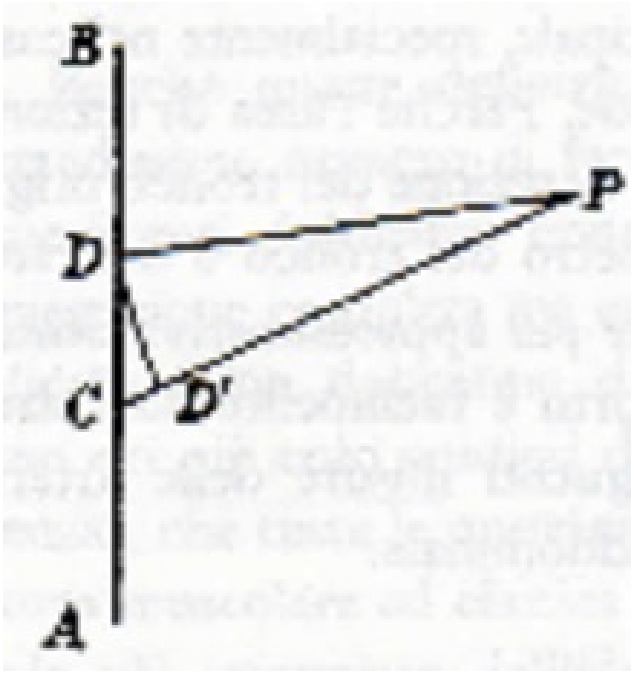


Figure 4 - Path ACP is compared to path ADP in the light of the optimality principle of minimum work (D'Arcy Thompson 1917).

In 1901[25] Thoma addressed one fundamental parameter describing the relationships between the trunk diameter c (Figure 2) and its branches a and b.

This relationship was defined by the equation:

$$c^{\Delta} = a^{\Delta} + b^{\Delta}$$

Δ being the diameter exponent parameter.

Following Thoma's identification of the diameter exponent, the story gets far richer since Thoma himself, D'Arcy Thompson (1917), Cecil Murray (1926[26]) and more recent works (Kurz et al. 1997) all tried to calculate its optimal value and to corroborate it with empirical observations[vi] [27] .

However, this is another story, to which Roux, whose interests soon turned to the initial developmental phases of cellular differentiation, did not make any direct contribution.

Until now, our analysis has stressed Roux's emphasis on the close matching between the anatomical regularities underlying vascular architecture and a condition of physiological optimality: mathematically described vascular forms (Roux's laws) reflect the best hemodynamically described vascular function (principle of minimum work).

However, the relationship between form and function can be intended as a relationship between an explananda

(the form) and its explanans (its function) with respect to two different questions:

- (1) why has the vascular system come to have such a regular branching structure?

- (2) how is this regular branching structure produced during development?

This duality corresponds to Mayr's (1961[28]) difference between ultimate (why? How come? questions) and proximate (how? questions) causes: (1) by stressing the physiological optimality of human vascular architecture, Roux seems to advocate that, once we assume a principle of minimum work as the proper criterion for efficiency, either the present vascular architecture has been selected for its optimal efficiency or different vascular architectures have been excluded because of their scarce efficiency. In other words, vascular regularities are evolutionary adaptations i.e. characteristics being selected for because of their fitness value. In the context of this former question, hemodynamical laws play the role of physical constraints: fluid mechanics set the basis and the limits for an optimal physiological condition as it defines the adaptive value of the vascular structure and in this sense, it is said to ultimately explain its existence. However, what is most important is that in this former question hemodynamical laws do not explain the developmental origin of vascular regularities; they are not rules of construction, only physical constraints on vascular functionality[vii] [29] .

If we move to the second question (2), addressing the issue of which originating proximate causes are responsible for the development of vascular morphology, we get closer to the difficult intersection connecting Roux's 1878 dissertation and his 1881 work on Functional Adaptation.

What are the proximate causes of vascular morphology? Roux offers two different but not necessarily conflicting answers. In 1878 "by making a parallel between vessel branches and the shape and direction of flowing water" he hypothesized that "blood pressure had a bearing on the patterns of branching" (Churchill 1973, p. 570), which in other words means there is a direct mechanical (hemodynamical) effect of blood flow on the shape and direction of the vessels. This explanation is purely mechanical (or to be precise hemodynamical) and remarkably in line with the later tenets of *Entwicklungsmechanik*: morphological rules should be reducible to physical mechanisms (hemodynamical forces) directly shaping the organism's structure. However, in 1881, Roux' morphogenetic explanation of the vascular structure underwent a curious reformulation through the introduction of the concept of functional adaptation. While in 1878 physical forces were sufficient to account for vascular morphogenesis, in Der Kampf Roux once more tackled the problem of functionality: the finest details of vascular structure are the result of an adaptive reaction of the organism to relevant stimuli occurring during development. If ever hemodynamical forces can explain the overall branched structure they are not enough to explain the direction and the thickness of spreading vessels: some adaptive (regulatory) mechanisms coupling organisms to environmental demands are necessary to explain the finest morphological details.

Functional adaptation and blood vessel branching

The concept of functional adaptation (funktionelle Anpassung) was introduced by Roux in his 1881 Der Kampft der Theile im Organismus and retrospectively used to explain, among other morphological phenomena, the developmental appearance of some seemingly "finalistic dispositions" of the vascular architecture. Blood irrigation of growing organs in normal development, of tumors in pathologies or of the fetus during pregnancy are, according to Roux, all examples of the capacity of the vascular system to react to developmental stimuli by rearranging its own structure. More generally, functional adaptation is the capacity of the system to change its form according to the requirements of a new function.

At the time Roux was writing, the concept of functional adaptation appeared to be a very close analogy to Lamarck's principle of the acquisition/loss and inheritance of morphological traits through the principle of use and disuse[viii] . However, before examining the relation between Roux's concept of functional adaptation and Lamarck's concept of acquisition and loss of characteristics, it is worth distinguishing between an original formulation of the "principle of use and disuse" by Lamarck himself and its fortune among his followers[ix] .

Lamarck believed that physical matter in living organisms was so organized as to functionally react to changes in the external environment (Lamarck 1802[30]), the term "functionally" meaning nothing more than the induced agreement between the environmental perturbation and the direction of the external change. He believed that such a reactivity could be ultimately explained as a material property of living beings, and thus did not depend in any way from anthropomorphic notions such as the "will", "habits" or "action" (Gayon 2006[31]).

However, the solid anchoring of Lamarck's theory in the material world of natural laws became a misleading point in its reception. According to the received view of Lamarck, best known as Lamarckism, the developmental matching of form to the newly required function could be explained through the aid of an inner disposition, not only a vital[x] but a finalistic cause directing the process of morphological change.

It is reasonable that Roux received Lamarck's ideas filtered through a Lamarckist reading. This would explain why he conceives his concept of functional adaptation as a denial of Lamarck's appeal to a finalistic cause, to which he opposes a Darwinian solution, but at the same time leaves the door open for what he considers to be Lamarck's inheritance of acquired characteristics (acquired, though, by non-finalistic processes).

In relation to the functional acquisition/loss of traits, the publication of Darwin's "Origin of Species" subverted the logic of the argument: species' acquisition of environmentally well adapted traits or loss of non-adapted ones during evolution, was the result of a process of natural selection acting on organisms showing random variation in their morphological traits. Traits acquired through a process of natural selection are called adaptations.

In the "Origin of Species" Darwin (1988[32]) aimed at explaining the diversity of species[xi] [33] as a result of adaptation to different external environments. He assumed that:

- despite heredity, organisms show random variation

- provided the ecological resources are not infinite, natural selection acts on organisms by favouring the reproduction of the fittest or by eliminating the less fit.

Unlike the theory attributed to Lamarck, no finalistic stance is included in Darwin's theory of species evolution.

As Heams (2012[34]) has cogently said, Roux saw the possibility to shift "from a teleological causality (the Lamarckist solution) to an historical one (the one aimed at through the reformulation of the Darwinian logic within the organism)", a shift which is also evident from the subtitle of Der Kampf "Contribution pour un perfectionnement d'une theorie de la finalité mechanique" where the term "finalité mechanique" makes reference to the possibility of identifying proper mechanisms causing apparently finalistic traits.

In the introduction of Der Kampf, Roux writes

"La finalité n'est pas une réalité volue mais devenue, pas une réalité téléologique mais historique, apparue de manière méchanique; car ce n'est pas ce qui correspondait à un objectif préétabli qui a sourvécu mais ce qui possedait les caractères nécessaires les plus à meme de permettre sa survie" (Roux, 2013, p. 30)

Though not formulated in physical-chemical terms (stricto sensu mechanistic), Darwin's natural selection could be understood as a lato sensu "mechanistic explanation" of evolutionary phenomena, opposing Lamarck's teleological (vitalistic) concept of an inner disposition.

However, Darwin's theory of natural selection on organisms was deemed unable to account for complex adaptations (e.g. the compound eye) that required the correlated variation of many functionally dependent traits. This objection, recognized by Darwin himself (1988) and further stressed by Mivart (1871[35]), was probably one of the weakest spots of the newborn evolutionary theory. Roux's concept of functional adaptation aims at filling precisely this gap: if Darwinian variation of traits is random with respect to the organism's functionality (and thus not correlated), functional adaptation is the precise mechanism for understanding infra-organism correlated changes during development in response to hereditary or environmental variation.

Roux speaks about an improvement of Darwinian theory ("Contribution pour un perfectionnement […]"), which by itself is not sufficient to account for the origin of such "fine grained finalistic dispositions": no combination of blind variations could originate such a functional outcome as the perfectly adapted, all-encompassing vascular branching. As in the case of the eye, pointed out by Darwin himself, the functional complexity of the vascular architecture also seemed hardly to be evolvable by random variation and natural selection.

What was needed, according to Roux, was a mechanism allowing for the functional correlation of infra-organism parts during development: whatever (hereditary or environmental) random variation had occurred (e.g. an increase in the number of appendages), there should be a mechanism able to accommodate the variation through a process of developmental rearrangement (e.g. vascular irrigation, muscle formation for the new appendages). Whether the effects of such a process were inheritable or restricted to the individual life span is highly controversial in Roux's work. Their persistence through generations would explain the evolution of complex traits: once a random (inheritable) variation has occurred, functional adaptation drives the organism to rearrange its internal structure accordingly while its trans-generational effects simulate the occurrence of inherited functionally correlated variation[xii] [36] [37] .

However, even if we are not so strict about the possibility of inheriting functionally adapted traits, functional adaptation is still a powerful concept for explaining developmental plasticity i.e. the capacity of the organism to react to internal or external variation to preserve a functional outcome.

In order to define "functional adaptation" mechanistically, Roux exploited Darwin's selectionist mechanism and tested its strength at the infra-organism level of molecules and cells.

That is the origin of the first[xiii] [38] [39] transposition of the Darwinian logic made up of random variation and selection from the external environment to the internal one: the first theory of internal selection[xiv].

The bulk of the argument is that as natural selection explains the origins of different well-adapted species, internal selection accounts for the origin of different well-adapted organism parts: evolution is mirrored by differentiation and growth.

This parallelism unavoidably reminds us of Haeckel's "biogenetic law" where the course of ontogeny is explained through phylogeny (the pattern of evolution). However, the huge difference between the two formulations is that Roux does not consider evolution to be an explanation of development but the explanatory mechanisms at stake in Darwinian theory to be coopted to explain development.

Initially, Roux states that variation exists at every level of the organisms: molecules vary with respect to their chemical duration, their assimilating capacity and their time of duplication. Variation in molecules and cells' assimilating capacities within a population trigger a selectionist process which allows, mechanistically, for the survival of the fittest.

Citing Weismann's interpretation of Roux' selectionist account of functional adaptation (1909, p. 247): "Just as in […] personal selection" (to be intended as natural selection) "variability and inheritance lead, in the struggle for existence, to the survival of the fittest, so in histonal differentiation" (to be intended internal selection) "the same three factors lead to the victory of what is best suited to the parts of the body in question". Further on Weismann points out "variability - in this case that of embryonic cells with different primary constituents - must be assumed; inheritance is implied by the multiplication of the cells by division; and the struggle for existence here assumes its frequent form of a competition for food and space" (ibidem).

When dealing with the law of dimensional hypertrophy of a muscle for example, selection of proliferating and/or growing cells can be accomplished through selection of those cells able to react to the external stimulus through the metabolic capacity of hyper-assimilation. This reactive capacity is itself a random variation affecting only a niche in the cellular population.

By postulating internal selection of responsive cells, Roux's theory explains how development can accommodate functional changes, with no need for inherited correlated variation to occur. In this way, he found a mechanistic (non-finalistic) solution to Lamarck's problem of ontogenetically acquired characteristics.

In order to understand the explanatory role of functional adaptation in the case of vascular structure, we should return to Roux's description of the vascular branching in terms of vessel angles, lumen, directions and wall thickness. As previously noted, in order for hemodynamical principles to play the role of constraints on functionality in Roux's laws, the point of arrival of the vessel (in Figure 4 point P) has to be already established.

Unless we make the hypothesis that the points to be irrigated are predetermined by the system, which means hereditary, we need a specific mechanism to identify areas requiring blood supply and this is precisely the role that Roux attributes to functional adaptation.

The boundary between hereditary and acquired traits, however, is not so easy to detect. Scientists tend, according to Roux, to mistake this difference between hereditary and acquired for the other well-known difference between congenital and post-embryological (after birth or hatching) (Roux 2013, p. 62). This amounts to mistaking a difference concerning the cause of the developmental origin of morphological characteristics for a difference concerning the ontogenetic time of their appearance.

Indeed, acquired traits (through the mechanisms of functional adaptation) cannot be relegated to the post-embryonic period because the interactions of the organism with the external environment start far earlier than its birth.

From this point of view, congenital blood vessel morphology - "la structure de leur paroi et la forme de leur lumière" (Roux 2013, p. 63) - may be the result of functional stimuli and all the more so because they already show metabolic activity at birth (unlike other organs such as those belonging to the respiratory system and the digestive tract).

Even if "we are not able to determine the extent to which traits are inherited or acquired by functional adaptation" ("nous ne sommes pas en mesure de determiner la part de ce qui est héréditaire et de ce qui est acquis par l'adaptation fonctionnelle") (Roux 2013, p. 63), the are some clues which can help us pointing to one or the opposite direction: hereditary traits for an organism, according to Roux, tend to show a fixed and defined development and they cannot be easily diverted from their path.

Roux considers regeneration of an adult snail eye as a perfect example of a hereditary characteristic. Once the experimenter has removed the snail's eye and segregated it in the dark so that no functional stimulus can influence the regeneration event, the reappearance of the eye is considered the result of some "internal properties of the part"[xv] [40] [41].

Differently, the origin of acquired traits is strongly dependent on functional stimuli[xvi]. Those traits can

be experimentally recognized because they vary their morphology in agreement with the amount or extent of external stimuli. One of the first examples introduced by Roux deals with the capacity of muscles to increase their size after prolonged use and more importantly with the fact that change is only achieved through a growth in their thickness and not in their length. Indeed thickness is what is required to perform a better function while an increase in length would make them more fragile.

He summaries this evidence through the "morphological law of dimensional hypertrophy" which states that organs "only develop in those dimensions which are required by the application of function" (Roux 2013, p. 41)[xvii] .

At this point of the story, a difference is worth noting. Roux identifies two different kinds of functional adaptation: the former is the property of active organs, which are able to increase -or eventually decrease- their size according to the variation of their activity. This is an active form of functional adaptation, which strictly depends from the existence of specific external stimuli inciting organs to react actively. Another form of functional adaptation is the one performed by the so-called passive organs, such as the vascular system and arguably the peripheral nervous one. According to Roux, "most of the structure and form of the blood vessels arises in direct adaptation to function […] the vessels of adult men and animals are not fixed structures, which once formed, retain their form and structural build unchanged throughout life. On the contrary they require even for their continued existence the stimulus of functional activity" (Oppel;Roux 1910, p.125[42]).

When speaking of a functional stimulus in the case of blood vessels, Roux speaks of an unspecific internal stimulus coming from an organ, which is itself actively engaged in a process of -active- functional adaptation. Blood vessels irrigate those organs that call for an increase in blood supply because of an increase in their activity. From this point of view, functionality is both externally driven during the development of organs thanks to differentiating environmental stimuli and internally driven during the development of the vascular system thanks to an increase in the activity other organs.

"*La formation des parties ayant un role passif depend du fonctionnement embryonnaire des parties ayant un role actif*" (Roux 2013, p. 66). This also means, according to Roux, that dysfunctioning (hypo-functioning) active organs (e.g. one kidney) will not be vascularized in the same way as normal functioning ones or that hyper-growing tissues, with a strong metabolic activity, will be properly vascularized, thus also supporting tumor proliferation.

A further point about passive organs concerns their possible dysfunction. Here Roux's focus is on vascular dysfunctions also known as angiomes. He speaks about plane and cavernous angiomes which are both attributed to a shift from dependent (functional) development to an independent (dysfunctional) one.

Thus, blood vessel branching is dependent on the functionality of other organs.

However, while there are detailed explanations of the mechanisms responsible for accomplishing the first type of functional adaptation - at the crossroads between organs and external stimuli - the same precision is lacking with respect to mechanisms of the passive type.

Here we will try to build a coherent framework without forcing the fragmented structure of Roux's argumentation. Blood vessel branching is about finding the causes of the vascular branching, which from Roux's point of view, deals with the proximate cause of specific vessel bifurcating angles, lumen, wall thickness and direction.

Similar to bifurcating angles, we could approximately say that lumen, wall thickness and vessel direction also obey to an optimality principle: "la distribution du sang dans l'organisme se produit avec un frottement minimum dans les innombrables embranchements, c'est à dire que la circulation est rendue possible avec un minimum de force vitale et de material parietal". Indeed Roux says that all those morphological traits (lumen, wall etc..) are extremely fine tuned to the metabolic needs of the organism. However, which "optimal" vascular morphologies are due to inherited developmental rules of construction i.e. hydrodynamical forces, and which are due to the developmental interplay between the organism and the environmental stimuli i.e. passive functional adaptation is an open question.

In other words, "optimality" may be the result of natural selection and may also be the result of internal selection.

Arguably, the only trait which seems to be shaped by functional adaptation is the direction of blood vessels: passive functional adaptation, explaining angiogenesis, can only be framed within the selectionist approach by substituting external stimuli with internal ones.

However, angles, diameters and vessel wall, though being optimal traits according to the hemodynamical constraints, are not directly explained by a mechanism of internal selection. It seems that Roux comes back to his 1878 hypothesis of a "direct moulding" of those structure by hemodynamical forces (Kurz et al. 1997).

Let us consider for example the vessel lumen at the bifurcating points: "Au dèbut de chaque branche, la lumière des vaisseaux sanguins ne se presente pas sous une forme cylindrique, comme c'est le cas au milieu des branches, mais sous une forme conique caractéristique. […] la lumière adopte librement […] cette forme, c'est à

dire sous l'effect à l'oeuvre à l'interieur d'elle". Here the hemodynamical role is not that of a posteriori functional constraints, which explains why, in terms of evolutionary adaptation, vessel branching has been internally selected to show this particular growth pattern. They are intended as physical forces in the same way as the hydraulic forces of a river shape its riverbed (e.g. "de la meme manière que pour un jet qui s'écoulerait librement" (Roux 2013, p.52).

However, if blood's flow shapes vessel lumen, vessels have to be there already. Their formation is partly subjected to hemodynamical laws, partly to passive functional adaptation. Primary directions are given by the composition of physical forces (flow speed and lateral pressure) while their final direction, towards points of irrigation, is obtained through functional adaptation.

As the reader will probably notice, there is a conundrum in Roux's argument: either he explicitly resorts to the principle of functional adaptation and explains the optimality of its outcome, or he calls for hemodynamical laws to be directly shaping the structure of the system without any need for an external stimulus. The main difficulty is that, even when speaking of hemodynamical laws, Roux maintains that the phenotypes produced are optimal thus confusing optimal developmental adaptations, the ones mechanistically produced by internal selection, with optimal evolutionary adaptation, due to the natural selection of highly efficient and inheritable vascular branching rules.

A resourceful mechanistic explanation

At this point of the analysis, it will be clear enough that the need for a "mechanistic explanation" is among Roux's major concerns: embryological explanations are said to be mechanistic type-a in op-position to Haeckel's phylogenetic ones; at the same time hemodynamical forces moulding vessel angles are said to be mechanistic type-b with reference to their reducibility to physical-chemical laws. Finally functional adaptation, through its process of internal selection, is a mechanistic type-c explanation of infra-organismic change in opposition to a teleological one.

"Mechanistic" is thus a "Πολυμηχανος" concept[xviii] synonymous with at least three different kinds of causal explanations: analytic (vs phylogenetic), physical-chemical (vs not yet reducible) and historical (vs teleological).

Coming back to Roux's 1878 and 1881 theories of vascular morphogenesis in the light of this epistemological clarification, we realize that both of them, hemodynamical forces and functional adaptation, are "mechanistic" but in two clearly different senses.

As far as hemodynamical forces are concerned, they are mechanistic type-a and type-b because they seek developmental factors producing vascular architecture and recognize those developmental factors to be physical laws of construction.

Roux seems to suggest that certain traits of the vascular architecture are the result of a self-organizing process based on purely physical-chemical forces acting on the embryological matter.

As far as functional adaptation is concerned, it can be said to be mechanistic type-a because it aims at explaining the developmental production of phenotypic traits but at the same time it is mechanistic type-c because it explains their adaptation to the external environment through a selectionist, thus non-finalistic process.

In this second case Roux seems to suggest that environmentally attuned traits are the result of an adaptive mechanism that, in order not to be teleological (cfr. Lamarck), is presumed to be a selective one. In today's developmental biology, we would probably place hemodynamical forces and functional adaptation under different explanatory labels. Roux's explanation, making use of hemodynamical laws, is very close to what we would call today a "structuralist approach". In developmental biology, structuralism has a long tradition from D'Arcy Thompson (1917) to Brian Goodwin (1990[43]) and more recently Stuart Newman (2003[44]); it is concerned with the explanation of developmental regularities through the aid of physical-chemical laws[xix] .

Conversely, Roux's concept of functional adaptation points to the existence of adaptive mechanisms, which allow the organism to tune its development with the functional stimuli coming from the external and internal (embryological) environment.

In today's developmental biology, this reactive capacity of the organism would better go under the heading of "developmental plasticity": among the manifold adaptive mechanisms, internal selection has been proved to play an important role in immunological responses and in neural development (Corbellini 2001[45]) but regulatory, genetically based or epigenetic mechanisms also play a major role[xx] [46] [47] .

Of course, Roux did not make himself explicit this epistemological differentiation between the different senses of "mechanistic" nor he could draw a clear distinction between a "structuralist" approach and one focused on the organism's adaptive capacity. The feeling we have while reading his work is that the notion of causality implied by hemodynamical forces gradually blends into the different notion of causality suggested by the use of the concept of functional adaptation. Moreover, this epistemological confusion is fueled by the fact that Roux sees both "structural" and "adaptive" traits through the lens

of optimality[xxi] , which in the former case is the result of developmental rules of construction while in the latter is the result of a process of infra-organism selection.

However, we must remember that his main objective was to show the scientific value of mechanistic explanations with respect to historical (Haeckel's phylogenetic necessity) and vitalistic (based on immaterial final causes) ones. Hemodynamical laws and functional adaptation, though being different kinds of mechanisms, fulfilled this task: the former, with a focus on physical-chemical mechanisms aimed at explaining vascular morphogenesis through material (vs immaterial) proximate (vs phylogenetic) causes. The latter, differently, aimed at unmasking the myth of finality as the result of a goal oriented immaterial force by introducing mechanisms of internal selection able to account for final dispositions as un-oriented adaptations. Thus though the main difficulty that we encounter in trying to encompass Roux's scientific thought about developmental causes is the conceptual mixture expressed by the word "mechanistic", we are bound to recognize that the ambiguity surrounding this word makes it an eminently resourceful concept.

Conclusions

In this essay, we have explored the work of the XIX century embryologist Wilhelm Roux (1850-1924) with particular focus on the role played by his research on the vascular system in the formation of his embryological theories.

In the "Introduction", we have outlined our epistemological analysis with respect to two of Roux's early works: his doctoral dissertation on blood vessel branching (1878) and his theoretical volume on functional adaptation (1881).

In section I "Wilhelm Roux (1850-1924) and the prelude to *Entwicklungsmechanik*", we have sketched out the epistemological background to Roux's academic formation: Darwinian revolution and biophysical research in physiology respectively shape the debates opposing historical to proximate causality and vitalistic causality to physical-chemical one.

In section II "Order in blood vessel branching: Roux's anatomical observations", we have introduced Roux's vascular observations: the identification of a regularity in vessel bifurcating angles and the verification that the whole vascular structure can be described through an optimality principle of minimal physiological work.

In section III "Functional adaptation and blood vessel branching", we have introduced Roux's embryological concept of functional adaptation, which is meant to be a transposition of the Darwinian logic of variation and selection inside the organism, and we show how Roux attempts to use it to explain the developmental constraints on blood vessel direction.

In section IV "Πολυμηχανος" mechanistic explanation, we have underlined the epistemological ambiguity of the key concept of "mechanistic" causality and notice how this prevents giving a perfectly coherent picture of Roux's thought in the two texts examined.

Acknowledgments

This essay was written during a three-year funded PhD at Sapienza University of Rome.

Endnotes

[i] The explanation of blood vessel branching through the concept of functional adaptation is not mentioned in Roux's 1878 doctoral thesis where the focus is on the explanatory role of hemodynamical laws. Indeed the concept of Func-tional adaptation was only introduced in 1880 in *Ueber die Leistungsfaehigkeit der Principien der Descendenzlehre zur Erklaerung der Zweckmaessigkeiten des thierischen Organismus* (Roux, 1895), later examined in *Der Kampf der Theile* and retrospectively applied to some aspects of blood vessel branching (vessel directions). I thank Silvia Caianiello for bringing this point to my attention.

[ii] On the coincidence of mechanistic and mechanical explanation: "the causal doctrine of the movements of part has been extended to coincide with the philosophical concept of mechanism" (Roux, 1895, p. 150).

[iii] In a passage from "Order and Life" (1936) concerning the contribution of Roux's Entwicklungsmechanik to the rise of a "true, non-dogmatic organicism", Joseph Needham clearly catches the difference between these two notions (stricto sensu and lato sensu) of causal explanation in biology. "The ideal axiom at the basis of all causality can only be stated in terms of the mathematical concept of function. Physical equations necessarily involve functions. But an important distinction must be drawn between mathematical and mechanical" (Needham, 1936, p. 25). Causal explanation, according to Needham, is a broad epistemological category in relation to which mechanistic (=mechanical) generalizations are a particular case. Coming back to Roux's developmental mechanics, Needham stresses Roux's distinction between explanations making reference to "complex component" relationships and explanations arising from "simple component'" relationships. The adjectives "simple" and "complex" respectively stand for the possibility or provisional impossibility of a physical-chemical reduction of those regularities. However, even if "the aim of Entwicklungsmechanik is thus the reduction of the phenomena to the smallest number of causal processes" (Needham, 1936, p.21) - that is relationship

between simple components- Roux was totally aware that such a level of fine grained analysis could not yet be accomplished (either technically, or theorically) during the nascent days of Developmental Mechanics and he himself performed many of his experiments at the coarse grained level of embryological "complex components".

[iv] G. A. Schwalbe's publications "Beitraege zur Kenntnis des elastischen Gewebes" (1876) (tr. eng. "Contributions to the knowledge of elastic tissues"), "Ueber das postembryonale Knochenwachstum (1877) (tr. eng. "On post embryonic bone growth") and "Ueber Wachstumsverschiebungen und ihren Einfluss auf die Gestaltung des Arteriensystems (1878) (tr. eng. "On shifts in growth and their influence on the formation of the arterial system") prove his interest for the mechanical relation between form (anatomy and growth) and function (physiology). Schwalbe was Roux's doctoral supervisor in 1878.

[v] The technique of wax injection is thought to have been established in the early XVII century by Jan Swammerdam (1637-1680), Frederik Ruysch (1638-1731) and Regnerus de Graaf (1641-1673) and furtherly refined through the centuries. For details on the history of this technique see Zampieri and Zanatta (2012). Further details on the technique used by Roux are available in section I "Methodik und Fehlerquellen" (tr. eng. Methods and sources of errors) of his dissertation.

[vi] It is worth noting that the diameter exponent parameter has been progressively extended to the analysis of other animals' branched vascular systems (es. mammifers) once more creating an interest - though differently from Haeckel's XIX century "biogenetic law"- for comparative and evolutionary analysis in morphological explanations (LaBarbera 1990).

[vii] A similar case of physical constraint on functionality is the role played by the gravitational field in explaining the "allometric scaling of bones in different sized animals" firstly pointed out by Galileo Galilei (1638 cited in Carter et al. 1991, p. 3). In this case "to have a comparable structural strength for their body mass" -the biomechanical rather than physiological optimal condition- "large animals would need bones which were thicker relative to their length than smaller animals. […] However" this "sheds no light on the means by which such scaling is achieved" (ibidem, p.3).

[viii] On the proximity between Lamarck's and Roux's concepts see Roux (2013, p. 32): "Au sujets des effects de l'usage et du non usage, auxquels nous ferons désormais référence par le concept d'adaptation fonctionnelle […]".

[ix] I thank Silvia Caianiello for highlighting the need to discuss this distinction. Indeed once that distinction is made, the opposition between Roux's and Lamarck's explanations of the organism's reactivity blends considerably and it could be fruitful to enquiry about their similarities (Silvia Caianiello, personal communication).

[x] Though an anachronistic term at the time, it is better to use the term "biological" in the sense of being restricted to living beings.

[xi] In the second half of the XX century many biologists and philosophers of biology started questioning the power of evolutionary theory as originally conceived by Darwin and further strengthened by his supporters from the 1930s to 1960s, to explain both the process of diversification within species and between species. Two different terms have been coined: microevolution to address evolutionary change within population (whose dynamics are formalized in the field of population genetics) and macro-evolution to address change between species and higher taxa. For a complete and highly readable text on the topic, see Gould (2002).

[xii] As previously outlined, Roux's own position concerning the inheritance of traits acquired by functional adaptation was fluctuating. Arguably, just a few years later, after having committed to Weismann's restriction of inheritance to cells making up the germinal line (Weismann, 1892, 1909), he would neglect the possibility of such a functional, inherently somatic inheritance.

[xiii] For an updated study on the fortune of the mechanism of selection in other contexts than population genetics, see (Heams et al. 2013, Caianiello 2013).

[xiv] Roux's transposition of the concept of natural selection at the developmental level is an attempt to rescue Darwinism from the resurrection of finalistic causes. It has to be pointed out, however, that the adoption of selection as a mechanism for complex adaptations during development displays great explanatory power at the levels of molecule and cell proliferation but it is less sharp with respect to tissue development where a mechanism for correlation is preferable with respect to a mechanism for competition.

[xv] Shortly afterwards regeneration would become a leit motif of embryological research at Anton Dohrn Marine Zoo-logical Station, in Naples (Morgan 1901, Sunderland 2010).

[xvi] The fact that the effects of functional adaptation could eventually be inheritable does not imply that we cannot distinguish between inherited traits and their first ontogenetic occurrence through functional adaptation.

[xvii] Together with this law, he introduces also "a physiological law of dimensional hypertrophy" and "a morphological law for dimensional atrophy".

[xviii] Tr. eng. "resourceful". Ancient Greek word quoted in the incipit of Homer's Odyssey to describe Odysseus's strategic nature.

[xix] It is worth noting that the same emphasis on physical-chemical explanation has a different meaning for Roux (against vitalistic causality), for D'Arcy Thompson (against the abstractness of heredity), for Goodwin (against the reduction of all phenotypic characters to evolutionary adaptations) and for Newman (against genetic reductionism). Structuralism is thus a label for a set of homogenous positions -the explanatory power of physical-chemical laws- that, nonetheless, support very different objectives.

[xx] Our post-cybernetic notion of regulatory mechanism (Rosenblueth et al. 1943) based on the concept of feedback was unknown to Roux. For him the concept of "regulation" had no mechanistic basis and has to be rejected as every vitalistic cause (see Oppenheimer 1967 for the later debate with H. Driesch on the concept of independent or "mosaic" and dependent or "regulative" differentiation).

[xxi] "The temptation to reduce the two kinds of optimality - the one based on the reactive plasticity of living entities, the other to strictly physical hemodynamical constraints- could also reflect the very early phase of Roux's research. He will only later admit that total reduction to physical-chemical laws is not yet viable, and biology must resign to work with complex components" (Silvia Caianiello, personal communication).

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VASCULAR RESEARCH VASCULAR BRANCHING

The Vascular architecture. Phlebosomes do they exist ?

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presented to: the Hemodyn2015, Nov 2015, Naples, Italy
submitted: May 18, 2016, accepted: Jul 11, 2016, EPub Ahead of Print: Nov 30, 2016, published: Dec 31, 2016
Conflict of interest: None

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Abstract The angiosome is a 3D structure which is perfused by a single perforating artery (arteriosome) and drained by a perforating vein (venosome). The concept of arteriosome is applied in plastic surgery and in the revascularization of ischemic limbs. Each venosome is also drained by longitudinal veins running in the subcutaneous layer. Accordingly, the concept of venosome cannot be applied in the field of the venous disorders of the limbs. The concept of phlebosome consider both paths of venous drainage.

Keywords angiosome, arteriosome, venosome, phlebosome

The integuments are perfused by perforating arteries running along muscular septa before piercing the muscular fascia. Each artery perfuses a limited area of skin surface with the underlying subcutaneous fat. This block is called "arteriosome". At the boundaries of each arteriosome are vessels connecting the peripheral arteries of neighboring blocks. The concept of arteriosome is applied in the daily practice by plastic surgeons since the mid of '90[1] and more recently in the revascularization of ischemic limbs[2-4].

A similar arrangement was described with respect of the drainage of the cutaneous and subcutaneous layers[1]. A perforating vein drains the plexuses of small veins running parallel to the skin surface into the dermis and into the subcutaneous layer (Figure 1).

These veins are valvulated and converge from all direction to form the root of the perforating vein. This vascular structure is more commonly stellate or medusoid in shape, similarly to saphenous tributaries at the Sapheno-Femoral Junction. In other body areas, the collecting veins are polarized in one direction, as typically in the lateral thigh (Figure 2).

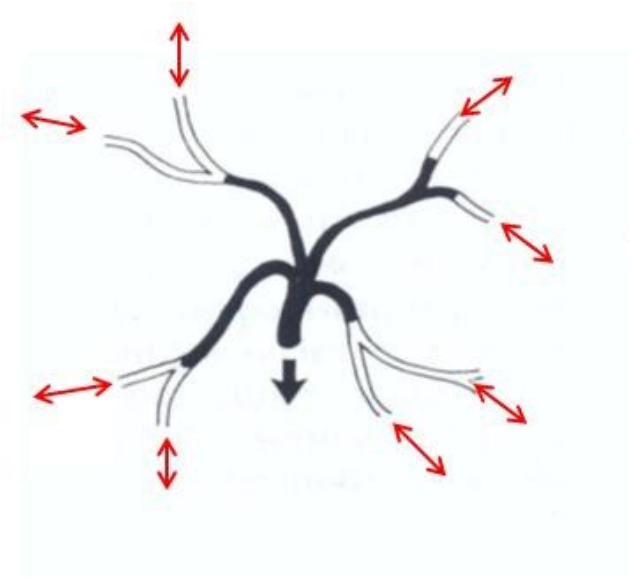

Figure 1 - bidirectional avalvulated veins in red.

Differences in the arrangement and polarization of collecting veins are related to the location, structure, function, embryological growth and differentiation[1].

The valvulated perforator drains the same block of cutaneous and subcutaneous tissues perfused by the corresponding perforating artery. This territory was named "venosome"[1]. At the boundaries of each venosome, avalvulated veins connect the peripheral valvulated vein

of adjacent blocks. These veins, were defined "oscillating veins" because *"..they allow free flow between the valvulated channels of adjacent venosomes, whose valves are oriented in an opposite direction.."*[1].

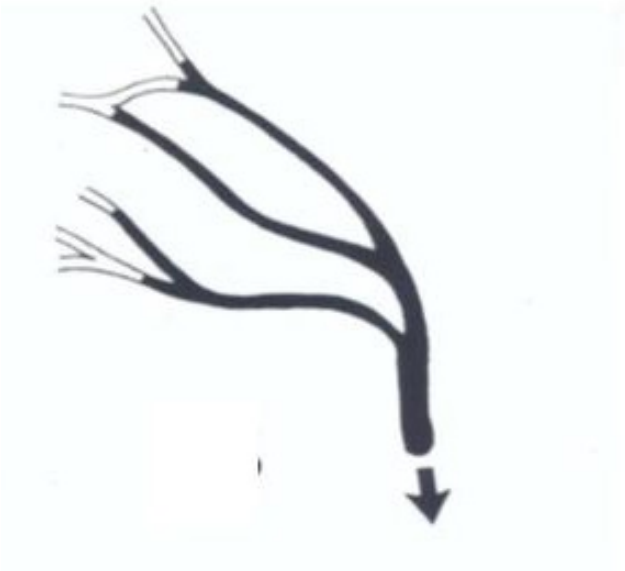

Figure 2 - The collecting vein polarizes blood flow toward deeper veins.

The subdivision of the integuments in venosomes is related to the arrangement of the venous system in the embryo: the limb bud is drained by perforating veins which confluence into the primordial cardinal veins. In the next phase of embryo development, veins running along the major axis of the limb develop in the subcutaneous layer: firstly the (medial and lateral) marginal veins, then the great saphenous veins with their parallel accessories.

These subcutaneous longitudinal veins play a predominant role in the drainage of the limb bud until the development of the deep ones, and actively participate to the drainage of venosomes . At the end of the first trimester of intrauterine life the deep veins develop (firstly the sciatic, than the femoral) and the hemodynamic role of the longitudinal subcutaneous veins progressively reduces.

However in the adult a double systems of blood drainage persists: a vertical system (saphenous accessories, saphenous veins, the lateral plexus) and a transverse system (perforators). (Figure 3) This is why, at least in the inferior limb, the vascular arrangement of venosomes does not mirror that of arteriosomes. Moreover, the hemodynamic changes observed in pathological conditions (i.e., varicose disease) are not ascribable to the simple architecture of venosomes. For this reason, the term "phlebosome" looks to better correspond to the venous architecture of the cutaneous and subcutaneous layers in the lower limbs. These structures are simultaneously drained by both perforating and longitudinal subcutaneous veins systems.

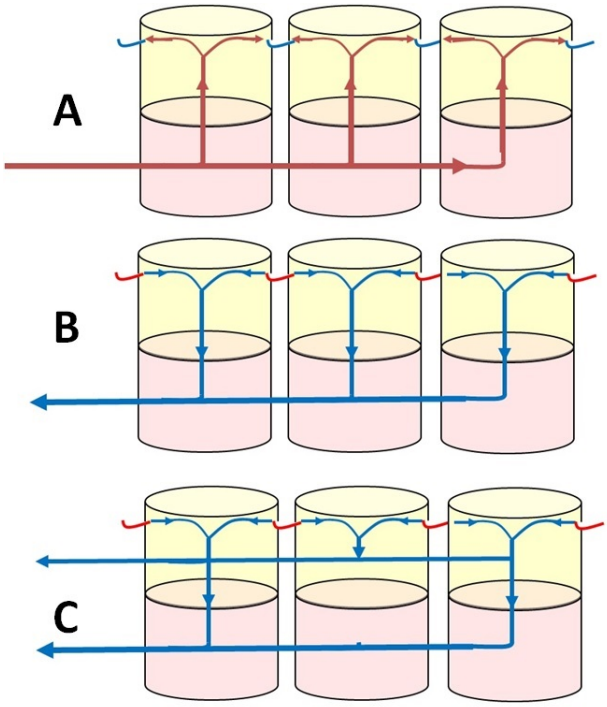

Figure 3 - A) the architecture of arteriosomes. Oscillating vessels in blu B) the architecture of venosomes. Avalvulated oscillating veins in red. C) the architecture of phlebosomes.

The peripheral arcades converge toward the respective perforator which pierces the muscular fascia to reach a deep vein. In other cases, it pierces the saphenous fascia to be drained by the saphenous veins. The peripheral arcades of each phlebosome are also connected by avalvulated veins to the peripheral ones of the neighboring phlebosomes (Figure 4). These avalvulated veins are responsible for equilibration of flow and pressure between neighboring phlebosomes.

In greater part of the phlebosomes of the inferior limbs it looks that the draining role of the perforator is overshadowed by that of the subcutaneous longitudinal vessels. This especially along the medial face of the limb and the posterior leg due to the presence of the saphenous veins. In greater part of varicose limbs, the venous changes are related to pathologic changes of longitudinal subcutaneous veins (Figure 5). Only in few cases, it is possible to observe a topographic relationship of signs and symptoms to a single "phlebosome": 1) the corona flebectatica, 2) the clusters of varicose veins originating from an incompetent perforator (escape point) and finally, 3) varicose vein of the lateral thigh drained by a lateral perforator.

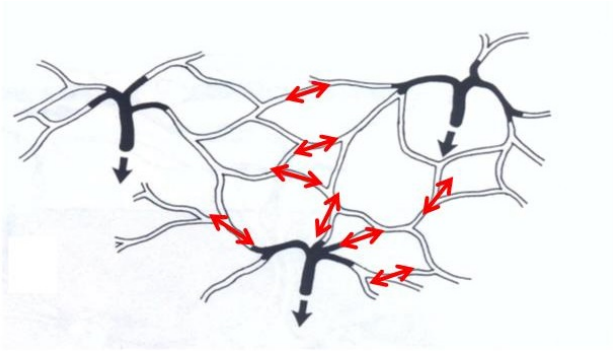

Figure 4 - Avalvulated oscillating veins connect the peripheral veins of neighboring venosomes.

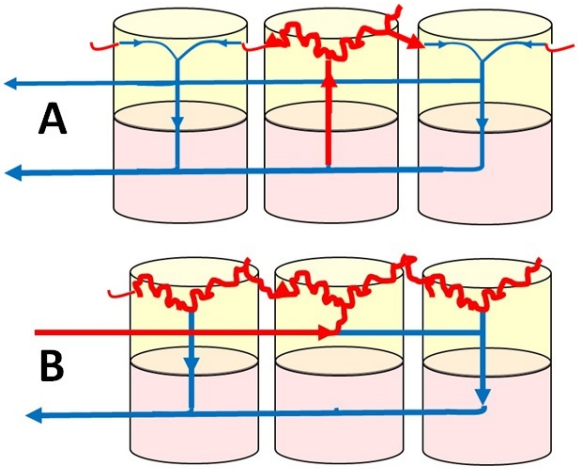

Figure 5 - A) reflux from an incompetent perforator with varicose veins limited to the related phlebosome. B) reflux from a longitudinal vein with varicose veins extended to same phlebosomes and drained by re-entry perforators.

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Theoretical approach of ductal morphogenesis

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presented to: the Hemodyn2015, Nov 2015, Naples, Italy
submitted: Jul 12, 2016, accepted: Sep 6, 2016, EPub Ahead of Print: Nov 30, 2016, published: Dec 31, 2016
Conflict of interest: None

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Abstract We developed 3D culture methods that reproduce in vitro mammary gland ductal morphogenesis. We are proposing a conceptual framework to understand morphogenetic events based on epistemologically sound biological principles instead of the common practice of using only physical principles. More specifically, our theoretical framework is based on the principle that the default state of cells is proliferation with variation and motility. We emphasize the role played by the agency of cells embedded in a gel and the circularity that is relevant for the intended process, whereby cells act upon other cells and on matrix elements, and are subject to the agentivity of neighboring cells. This circularity strongly differs from classical linear causality. Finally, our approach opens up the study of causal determination to multilevel explanations rather than to reductive ones involving only molecules in general and genes in particular.

Keywords Morphogenesis, extracellular matrix, theoretical principles, default state of cells, modelization.

Introduction

The proper understanding of epithelial morphogenesis and aberrant morphogenesis, for example in the case of cancer, requires an appropriate theoretical framework. Such a theoretical framework should take place within a theory of organisms that encompasses life cycles. This theory aims to provide a framework for both experiments and mathematical modeling of organisms and their parts. The role of theory that we describe here is similar to an extent with the manner in which classical mechanics and its concepts of space, time and forces frame the movement of classical objects in classical mechanics.

Theoretical descriptions in terms of genes or individual categories of proteins do not include a notion of space and time. As a result they are not sufficient to explain biological shapes because shapes are about space and morphogenesis is about space and time. Moreover, a theoretical framework for organisms should enable us to understand reciprocal interactions and the non-linearities relevant to morphogenesis. Therefore a good theory should enable us to perform mathematical modeling and interpret the meaning of the use of mathematics.

In order to develop a theory of organisms, physical theories are an obvious reference and it is so in two distinct manners. First of all, biological objects are surely compatible with physical theories when they are in their range of application; however this does not imply that current physical theories are sufficient to understand

biological organisms. For example, the (mass times) acceleration of a cell is surely equals to the sum of the forces that applies to it; however these forces are largely the result of an evolutionary and ontogenetic history, and handling this history theoretically requires an appropriate framework[1,2]. Second, physical theories are also a methodological reference, especially in their use of mathematics to understand natural phenomena. Third, in physics we witness that phenomena at different scales are explained by different fundamental theories such as cosmology, thermodynamics and quantum field theories. This experience in physical theorizing goes against overly reductionist positions often defended in biology. Again, physics being a reference does not mean that we must build a theory of organism following the same epistemology. In fact, we have contrasted numerous times the two domains, see in particular[1,3,4].

In this essay, we will present some elements of context on epithelial morphogenesis. In particular, we will emphasize the role of the stroma on the basis of experimental results. We will then present a model of cell culture in 3D collagen gels where morphogenesis takes place in a similar manner than in mammary glands in situ. Last, we discuss how we frame the situation theoretically and emphasize the manner in which we discuss cellular behaviors.

Stroma and morphogenesis

Morphogenesis, and more generally development, should not be conceived as the unfolding of a program contained in specific parts but as a result of the interplay of parts. Among them, we would like to emphasize the reciprocal interactions between the stroma and the epithelium. These interactions are of biophysical, in particular biomechanical, and biochemical nature, and the two aspects are often coupled, for example when a chemical compound induces changes of the cytoskeleton organization and the latter feeds back on gene expression[5]. Let us now discuss a few experiments which exemplify these reciprocal interactions and emphasize the crucial role of the stroma.

In an experiment[6], biologists transplant epithelium from the mammary gland in the stroma of salivary glands and in the other way around. The outcome of these transplantation experiments is that the morphology of the epithelium is determined by the stroma, so that the mammary epithelium in the salivary stroma takes the morphology of a salivary gland (and the other way around).

The extracellular matrix participates in the determination of cellular behaviors. Naive mesenchymal stem cells differentiation depends on the mechanical properties of their micro-environment[7]. More precisely, a soft matrix is neurogenic while a very stiff one is osteogenic. An average stiffness is myogenic.

Now let us consider a highly pathological morphogenesis: i.e., carcinogenesis. In an experiment[8], biologists expose animals to a highly carcinogenic compound: NMU. Then they perform all the possible recombinations of exposed and non-exposed stroma with exposed and non-exposed epithelium. The results show in very clear manner that an exposed stroma is sufficient to lead to tumor formation while an exposed epithelium recombined with a non-exposed stroma leads, instead, to normal morphogenesis. Let us also remark that metastasis is facilitated when the metastatic epithelial seed carries stroma, including fibroblasts[9].

This short discussion shows the importance of the stroma and more generally of the micro-environment in the determination of both the morphogenetic process and of cellular behaviors. The biological model described below is another relevant example of these events. We emphasize the effects of the extracellular matrix of the stroma on the epithelium among their reciprocal interactions because these effects are generally underestimated. However, we will see below that it is really their reciprocal interactions that enable us to understand a specific biological situation.

Biological model

Collagen-I is the main protein of the stromal extracellular matrix of mammary gland. A 3D biological model of mammary gland morphogenesis consists of a collagen gel containing epithelial cells. More precisely, collagen fibrils remain liquid at low temperature and they form a gel at ambient temperature, which can capture cells in suspension.

This in vitro model enables us to partially disentangle the relationships between our object of study and the rest of the organism. More precisely, this model makes it possible to control precisely the mechanical properties of the extracellular matrix and the cell type that participate in morphogenesis. Seeding cells of the breast epithelial cell line MCF10A in such gels leads to the formation of elongated epithelial structures, polarization of cells within the structures and the formation of lumen, which are then similar to mammary ducts[11] (figure 1).

Matrigel is a mixture of basement membrane proteins that can be added to collagen to obtain a gel with different properties than pure collagen gels. In a gel with 1mg/ml collagen and 50% (v/v) matrigel, MCF10A cells form spherical structurese close to the acini in vivo. The morphogenetic process with matrigel is thus distinct from the one without matrigel. With less matrigel (5%), cells form both spherical and elongated structures as illustrated in figure 1.

The bulk mechanical properties of gels are not sufficient to explain these different morphogenetic processes because gels with collagen and 0% or 5% matrigel have very similar macroscopic mechanical properties but lead nevertheless to different epithelial shapes[10]. However, these different gels have very different microscopic properties, as shown by electron micrographs. Collagen gels are fibrilar; they form a mesh of collagen fibrils. By contrast, the addition of matrigel leads to a more globular matrix, where fibers are less accessible, depending on the amount of matrigel added.

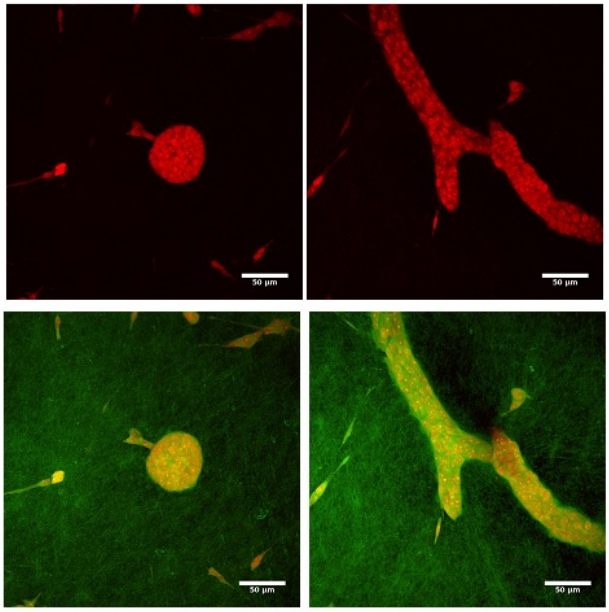

Figure 1 - Epithelial structures observed by confocal microscopy. The images are a projection of 3D stacks. In red, we observe cells by carmine staining. In green, we observe collagen-I fibers by reflective confocal microscopy (see also figure 2). All images come from the same collagen gel with 5% basement membrane protein (matrigel) which is known to lead to two kinds of structures. On the left side, cells from the MCF10A breast epithelial cell line form an acinus. On the right side, instead, they form elongated structures similar to ducts in vivo. Note that in the latter, we can see two elongated structures which are not connected, even though they are partially superposed. The experimental protocol to obtain these structures is described in Barnes et al.[10].

In collagen gels, cells reorganize collagen and do so very early, i.e., a few hours after seeding[10] (figure 2). Moreover, a macroscopic mechanical strain leads collagen fibers to be oriented along the direction of the strain and to the formation of elongated structures along the same direction[13]. Forces exerted by cells on collagen can affect distant cells at a range far greater than cell sizes[14].The inhibition of remodeling by the matrigel-containing globular matrix is central to the formation of acini. As a conclusion, we hypothesize that the interplay between cells and the matrix is central to morphogenesis, and more specifically, to the remodeling of the fibrilar matrix of collagen.Note that it is possible to refine this biological model to include other cell types such as fibroblasts or to change the initial conditions of the cells, or the mechanical constraints on the gel.

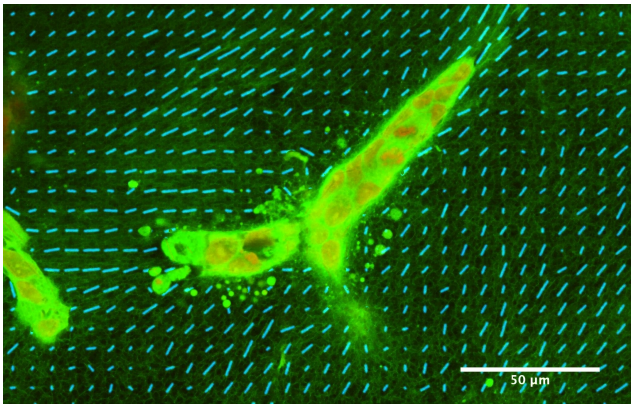

Figure 2 - Collagen orientation around an epithelial structure. Here, we represent in blue the average orientation of collagen fibers for each element of an arbitrary grid, using the method described in Rejniak et al.[12]. Collagen is oriented in the same direction than the tip of the elongated structure. This image exemplifies the fact that epithelial cells remodel collagen during the morphogenetic process.

Theoretical principles

One crucial difficulty in modeling biological systems is the lack of established theoretical principles to frame cellular behaviors. Cells are sometimes informally considered to be stimulated by "signals", which are usually chemicals and would "command" them to behave in a certain way, for example, by making them proliferate or move[12,15]. In other models, cellular proliferation or motility are assumed to be spontaneous but may be prevented by constraints such as a lack of available space for proliferation[12,16]. Sometimes, the different viewpoints are discussed for the same object without being explicitly articulated. Another viewpoint from physics proposes that cells are in a configuration of minimum energy; however, this idea cannot be considered as a general principle since cells are active entities which can and often do escape minimum energy configurations by the consumption of chemical free energy.

This brief overview shows that a general theoretical frame to understand cellular behavior is missing. In

previous papers[2,17] we have proposed to build such a frame on the basis of an analogy with classical mechanics. In the latter, a fundamental principle, the principle of inertia, specifies the behavior of a system when nothing is done to it. We call such a reference behavior a default state. Thus, te default state is the behavior of a system when no specific external cause acts on it. Assuming a default state is a fundamental principle for a theory. Reciprocally, causes, that is to say physical forces in classical mechanics, explain a departure from the default state. Epistemologically, a default state does not require an explanation; it is postulated and only departures from the default state require explanations.

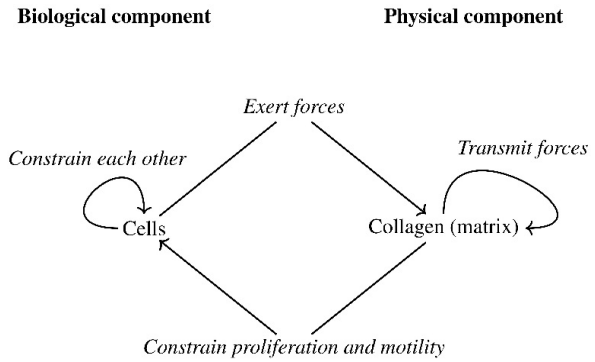

Figure 3 - Diagram of the different interacting elements of the biological model. We separate the components of the system on the basis of the principles that provide the theoretical framework to describe them. Classical mechanics and chemistry for collagen fibers, for example, and our default state for cells. These two kinds of elements are in continual interaction.

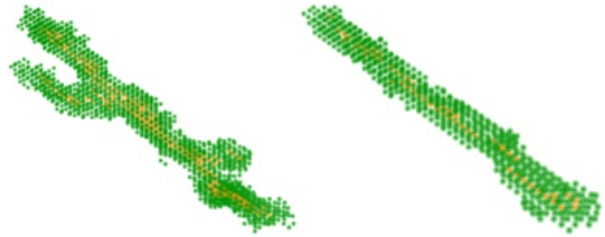

Figure 4 - Two different runs of a simulation of 3D cell culture of breast epithelial cells in collagen gels. In green we represent cells and in orange we represent the lumen. The model is described in Montévil et al.[21]. Note that branching occurs in the first run (left) while it does not on the second (right). These different behaviors stem from different values in the parameter representing the ability of cells to remodel collagen.

In line with former work[2,17-19], we propose that the default state of cells is proliferation with variation and motility. This entails that no cause is required to justify that cells proliferate. Instead what requires an explanation is quiescence. Similarly, we assume that motility is spontaneous. Note that motility is not restricted to cell mobility. Motility also includes, for example, the motion of cellular pseudopods involved in the reorganization of the extracellular matrix.

In our framework, the departure from the default state is the result of constraints such as physical forces, the attachment to other cells, chemical inhibitors [20], etc. Note that this framework is at least partially compatible with several existing mathematical models[12,16] and transforms local hypotheses into a general principle.

The systematic use of our default state in order to understand the biological model described in the previous section is the object of a paper in press[21]. Basically, we analyze this system as the interaction between two types of objects. The first is the collagen (or more generally, the extra-cellular matrix) which is determined by physical principles. In particular, collagen initially self-organizes in the process of gel formation. Afterwards, collagen transmits forces exerted by cells, i.e., during the process of morphogenesis. On the other side, the system includes cells which require a proper theoretical principle and we frame their behavior with the default state. Then, the analysis of the situation of interest corresponds to assumptions on the specific constraints that act on the default state and which stem mostly from the extra-cellular matrix and from other cells. The full system is then modeled as the interplay between these two components (figure 3). In figure 4, we propose two simulation outcomes that lead to elongated structures[21]. These structures result from the forces exerted by cells on the fibrillar matrix. By generating and applying these forces epithelial cells organize the collagen around them and constrain each other. These constraints lead to the formation of a dominant direction which is a typically non-linear phenomenon. In particular, cells that are in the middle of the tube cannot migrate or proliferate towards the exterior mostly because of the mechanical strain along the direction of the tube.

Conclusion

In this brief overview on mammary gland epithelial morphogenesis, we emphasize the role of the hypotheses and principles that we use to understand a system. We illustrate the idea that the interplay between the stroma, here represented by the extracellular matrix, and the epithelium is a crucial determinant of epithelial morphogenesis, including pathological morphogenesis as in the case of cancer. We also emphasize the need of a proper theoretical principle in order to frame cellular behaviors both in experimental and mathematical work. We present the idea that the default state of cells is proliferation with variation

and motility which is an evolutionary sound approach to frame cellular behaviors theoretically. The application of these ideas to specific mathematical modeling is the object of ongoing work and a first model has been published[21].

This work exemplifies the idea that fundamental biological explanations do not always need to appeal to the molecular level. Our principle builds on cell theory and thus consider cells as elementary but complex objects in order to understand tissues and their genesis. The default state of cells is a fundamental principle in the sense that the default state defines a causal structure: the default state does not need to be explained while a departure from it requires a causal explanation.

Acknowledgements

This work was conducted as part of the research project 'Addressing biological organization in the post-genomic era', which is supported by the International Blaise Pascal Chairs, Region Ile de France. AMS was the Blaise Pascal Chair of Biology 2013-15 at the École Normale Supérieure. Additional support was provided by Award Number R01ES08314 (P.I. AMS) from the National Institute of Environmental Health Sciences. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. Maël Montévil is supported by Labex "Who am I?", laboratory of Excellence No. ANR-11-LABX-0071. CS is a member of the Centre Cavaillès at the École Normale Supérieure, Paris France.

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VASCULAR RESEARCH VASCULAR BRANCHING

3D modeling of the vascular system

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presented to: the Hemodyn2015, Nov 2015, Naples, Italy
submitted: Apr 30, 2016, accepted: Jun 19, 2016, EPub Ahead of Print: Nov 30, 2016, published: Dec 31, 2016
Conflict of interest: None

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Abstract The 3D modeling of the vascular system could be achieved in different ways: In the **venous location**, the morphological modeling by MSCT venography is used to image the **venous system**: this morphological modeling tool accurately investigates the 3D morphology of the venous network of our patients with chronic venous disease. It is also a fine educational tool for students who learn venous anatomy, the most complex of the human body. Another kind of modeling (mathematical modeling) is used to simulate the venous functions, and virtually tests the efficacy of any proposed treatments. To image the **arterial system**, the aim of 3D modeling is to precisely assess and quantify the arterial morphology. The use of augmented reality before an endovascular procedure allows pre-treatment simulation, assisting in pre-operative planning as well as surgical training. In the special field of **liver surgery**, several 3D modeling software products are available for computer simulations and training purposes and augmented reality.

Keywords 3D reconstruction, vascular modeling, Multislice CT, Angio-CT, Volume rendering (VRT), vectorial modeling, Virtual reality, Simulation, Surgical training, Augmented reality, Educational anatomy

What is vascular modeling and why would we use it? Vascular modeling is a new tool which enhances the knowledge of anatomy and physiology of the vascular system and simplifies the planning of vascular interventions.

Different modalities of vascular modeling

1 - *When considering the VENOUS SYSTEM* the 3D modeling could have several objective:

1.1 The morphological modeling is used for

1.1.1 **Investigation by multislice CT venography** (MSCTV) of patients with chronic venous disease[1-8].

This modeling is very useful in cases of recurrent varicose veins after surgery, especially in the popliteal fossa, in patients with complex venous anatomy and with venous malformations. In all such cases it is essential to obtain additional venous hemodynamic mapping[9] by color Duplex Ultrasound.

But MSCTV provides more accurate morphological 3D information on the whole venous network, in particular of the deep system. Hence we think that MSCTV should be considered as a powerful navigator for varicose vein surgery, providing a complementary road-map for surgery[9,10]. For this purpose, 3D reconstruction is achieved by the means of a volume rendering technique (VRT) with the help of Osirix® software[11].

1.1.2 **For educational venous anatomy**:

3D modeling is a fine educational tool for the students who want to learn venous anatomy, the most complex of the human body[5]. It has a number of applications in the field of morphological sciences, in addition to the classical techniques of anatomical dissection. It is also a new and powerful research tool[6]. In order to develop these new tools and to create partnerships between Universities, we have created in 2015 with Pr Vincent DELMAS (chairman) a UNESCO chair of digital anatomy. For more information see the website: http://www.anatomieunesco.org/. In fact, the best way to build these 3D anatomical databases is by experts in anatomy, not computer graphics.

For this purpose, an anatomical segmentation is necessary before building the 3D vectorial models. This is done with the help of dedicated software programs[12,13] The aim is to build interactive computer atlases of the human body, in particular by the use of anatomical slices[14,15].

The handling of theses realistic 3D models provides a new educational tool for students: the virtual dissection table[16].

For the daily practice of phlebologists and surgeons, Interactive 3D venous atlases are particularly useful in understanding the complexity of venous anatomy, and the huge number of anatomical variations. In practice, improvement of our anatomical knowledge will improve the quality of our venous maps, which is the keystone of decision making and treatment guidelines[9].

1.1.3 **Modeling for simulation in liver surgery**:

3D modeling of the vessels and reconstruction of the liver segmentation[19] is soon to be introduced into selected surgical disciplines, in particular hepatic surgery

The *aim of* 3D modeling of the liver includes computerized simulations for training purposes, and augmented reality[17,18].

These new computer tools interact with medical images for surgical planning[19,20] and training, to assess surgeons' fitness to practice. 3D models may also guide micro-robots undertaking minimally invasive liver or abdominal interventions.

1.2 **Mathematical modeling** of the venous system has a totally different objective: to simulate the global venous function[21,23], in order to virtually assess the benefits of proposed treatments[24,25] of the chronic venous disease: it is a software called "venous return simulator" (VRS)

1.3 **CAAD (Computer Assisted Anatomical Dissection)** is a new research tool enabling a 3D reconstruction of the human embryo's venous system. This technique was originally used to study the anatomy of the pelvic nerves[26-31], but it is very accurate for the embryo's venous system[32-33].

2 - *When considering THE ARTERIAL SYSTEM*;

3D modeling is used to precisely assess and quantify the arterial morphology, particularly the use of augmented reality before endovascular procedures[34,35] Semi-automatic vessel segmentation is now possible with special software packages[36,37]. This provides (makes possible) a simulation of endovascular surgery, aids in pre-operative planning and surgical training[38] and allows for a rehearsal before operations[39].

The Techniques of vascular modeling

1 - Morphological modeling of the vascular tree:

Two different techniques are available for 3D reconstruction: Volume rendering (also called VRT) and vectorial modeling (also called surface rendering)

1.1 - Volume rendering or VRT (using digital data from multislice CT-venography[1-8])

The main steps of direct Volume Rendering (VRT) technique[1-3] are data acquisition, reconstruction, and post-processing: (Figure 1)

Data acquisition: A multislice and multidetector CT scan (64 and now 128 detectors) is used, producing 600 to 1200 slices by series over about 30 seconds.(see Table I for detailed protocol).

The timing of injection is critical: The contrast injection should begin about 40-60 seconds before, and then has to be synchronized with, the acquisition, in order that it finishes at the end of the acquisition.

The patient is lying supine (on his/her back, feet first), with no contact point with the table except for the buttocks and heels: It is important to avoid any compression of the calf and posterior thigh during acquisition time. The patient has to hold perfectly still during this short time and is often asked to do a Valsalva maneuver.

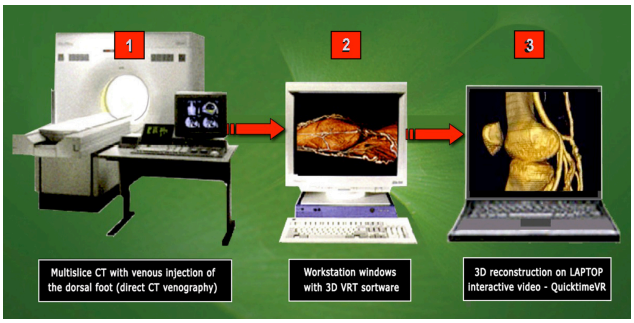

Figure 1 - The 3 main steps of the technique of direct multislice CT venography (MSCTV). 1 - Multislice CT with injection of the dorsal foot (direct CT venography). 2 - Workstation windows with 3D VRT software. 3 - 3D reconstruction on LAPTOP, Interactive video QuickTimeVR.

PROTOCOLS	ACQUISITION	RECONSTRUCTION	POST-PROCESSING	CONTRAST INJECTION
16 Detectors CT : 600 slices in 25 sec	120 kV 150 mAs slice collimation : 16 x 1.5 mm field 512 F.O.V 380 mm	slice width 2 mm slice increment 1.5 mm filter B30 matrix 512x512 zoom factor 1.7	Volume rendering (VRT) fast & automatic with tissues transparencies	- Medrad MSCT injector system. - single-phase injection 20 ml of iodine contrast medium in 180 ml of serum. - puncture of a vein of the dorsal foot or scarcely the varices of the thigh.
64 Detectors MSCT 1000 slices in 20 sec	120 kV 150 mAs	slice width 1 mm slice increment 0.75 mm matrix 512x512 zoom factor 1.7	VRT	
128 Detectors MSCT 1000 slices in 10 sec	100 kVp slice collimation of 128 × 0.6 mm	Rotation time 300 ms using a continuous helical scan mindose® technique pitch =0.16-0.22	VRT with PC using multi processors Osirix® using fast graphic card	Proximal injection & biphasic injection to visualize pelvic veins.

Table I - Multislice CT protocols. CT=computed tomography MSCT=multislice CT FOV=field of view VRT=volume rendering.

Data reconstruction: Raw data are processed to perform slices reconstruction. See usual protocols on Table I, from 16 to 128 detectors.

Post-processing of the data: 3D reconstruction of the venous system is performed. The data are usually sent by intranet on a dedicated workstation for post-processing using dedicated 3D reconstruction software. Then, the 3D interactive movies could be exported to a laptop.

The principle of VRT technique is to use a look-up table (LUT) establishing a correspondence between the levels of density and the colors (figure 2). A threshold selection makes it possible to isolate some

anatomical elements according to their specific density: skin, adipose tissue, muscles, vessels (injected) and bones.

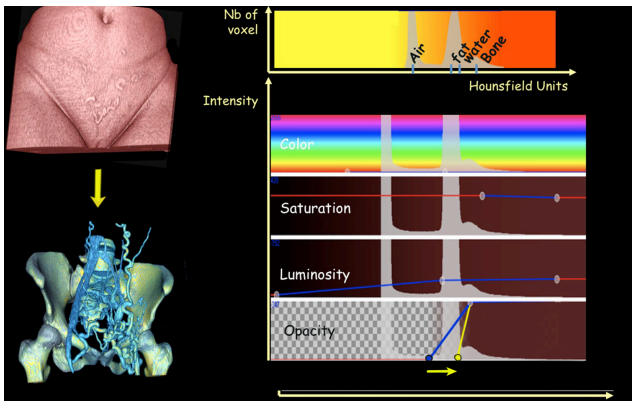

Figure 2 - The principle of Volume rendering technique (VRT) is to select a threshold in order to select a sample of densities in the histogram corresponding to specific anatomical structures. See the arrow on the right modifying the opacity, which makes the skin and muscles transparent (lower image on the left).

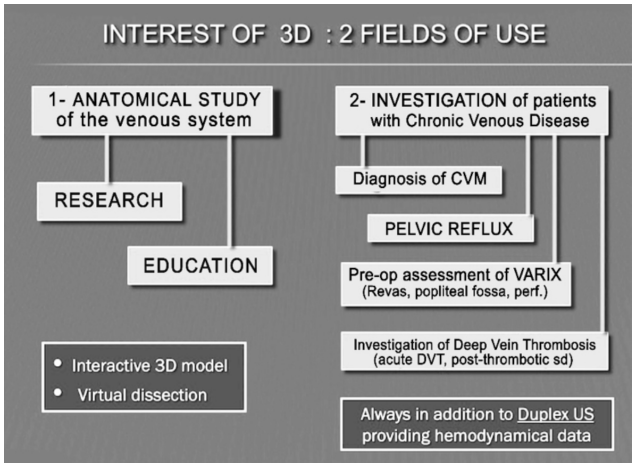

Figure 3 - The 2 fields of use of 3D modeling of the venous system by MSCTV.

Dedicated software can produce a realistic 3D reconstruction of the anatomy: Osirix® for mac computer[11] - Volume wizard, Syngo VRT (Siemens), Voluson 4Dview (GE), Philips, Infinix-i Volume Navigation (Toshiba).

Advantages of VRT are automatic and quick 3D reconstruction with threshold presets and automated vessel segmentation, but it needs powerful computers to handle the huge data sets.

Main indications of MSCTV[3,4] in the chronic venous disease (CVD) are shown on figure 3.

Results[5,8]: an example of 3D reconstruction of the venous network in the case of a patient suffering from pelvic venous reflux (Figure 4)

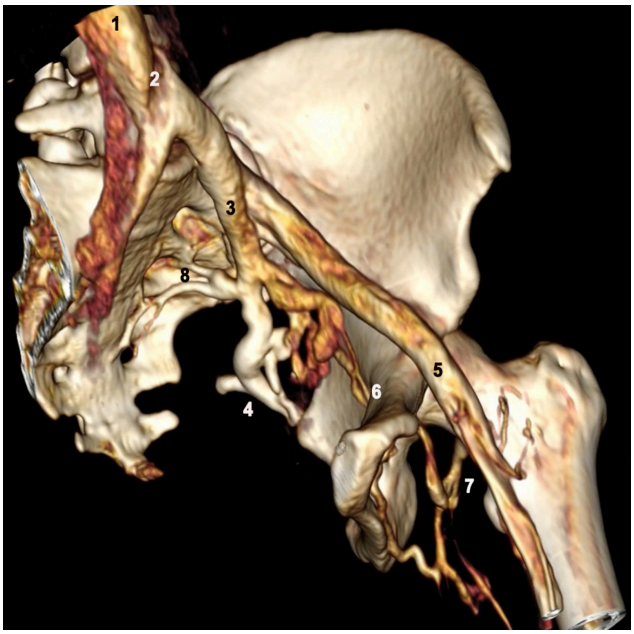

Figure 4 - CT-venography with 3D reconstruction by VRT. This woman has a pelvic reflux from the obturator vein (6) and a pseudo May-Turner syndrome (2). 1= inferior vena cava 2=imprint of the right common iliac artery 3= internal iliac vein 4=inferior gluteal vein 5=left common femoral vein 6=obturator vein 7=posterior circumflex vein 8=sacral plexus.

1.2 - Vectorial modeling[6] (surface rendering with ray-tracing)

For this we can use analogic data (for example anatomical slices), but a segmentation (by manual or semi-automatic) of the anatomical structures is required.

-Winsurf® software[12] (previously named Surfdriver) could be used to build the vectorial model. An example is given of the software screen during anatomical segmentation of the liver (Figure 5).

-Segmentation with Photoshop® and then use of Mimics® software (http://biomedical.materialise.com) has been used by the team of the Korean Visible man (http://vkh.ajou.ac.kr/#vk)

-Model display uses special CAD software (3Dstudio max® , Cinema4Dxl® , Autocad® , Amira® , Blender® , Maya® …)

-Conversion from winsurf® into Acrobat® 3Dpdf file format[13] enables interactive manipulation of 3D anatomical models.

-Results: creation of educational interactive atlases of anatomy and simulation.

This leads to the use of an *interactive virtual dissection table*: a computerized table lets the students easily do virtual dissections of the human body. It is a powerful tool for training medical students: a stretcher-sized multi-touch screen of the human body that lets you explore, dissect and understand the body's parts and systems.

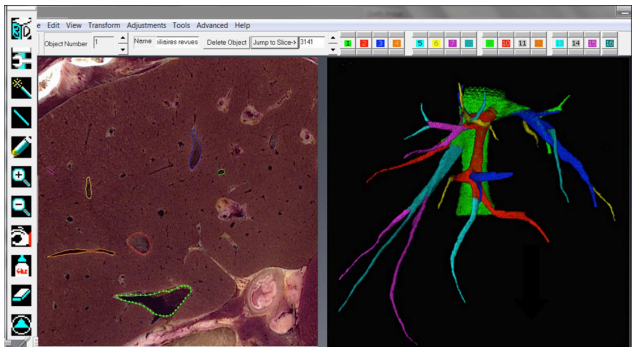

*Figure 5 - Interface window of the **Winsurf® software** showing the colored segmentation of the hepatic veins on the anatomical slice (Korean visible human) using the color channels. The resulting 3d vectorial model is shown on the right (including color channels).*

While Anatomage® (www.Anatomage.com) uses an expensive device and sophisticated software, the virtual dissection could also be achieved with a big touch screen running with Acrobat® 3dpdf models in a simple way[13] (figure 6). The big advantage is that the Acrobat® reader software is available for free on any computer.

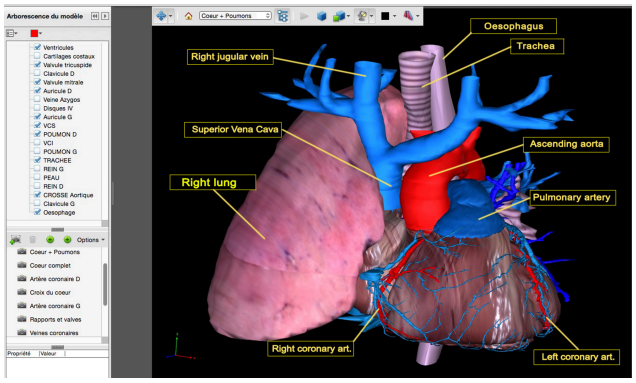

*Figure 6 - Interface window of **the Acrobat® reader software** showing the final 3D model of the heart and lungs which could be handled interactively with the labels. Each anatomical structure (on the left) could be displayed separately.*

1.3 - Vascular modeling for LIVER SURGERY has several useful applications:

-Surgical planning in hepatic surgery[14]

-Augmented reality for minimally invasive liver surgery[15]

-Planning of liver surgery for hepatocellular carcinoma (Figure 7)

-Hepatic transplantation planning.

-Visible patient planning (IRCAD) (Appstore for ipad, iphone)

-VP Planning is a computer software for Mac and PC to help surgical planning (https://www.visiblepatient.com/fr/)

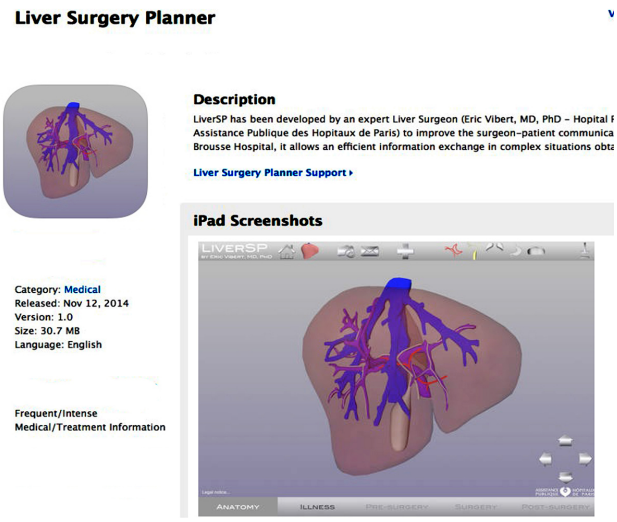

*Figure 7 - Description of **the ipad software "Liver surgery planner®"** (Dr Eric Viber, Paul Brousse hospital, Villejuif).*

2 - Mathematical 3D modeling of the venous network for simulation[16-19]. The aim is to build a virtual model, the venous return simulator[16] (VRS), designed to compute the venous hemodynamic variations when a compression device is applied on the leg.

The Venous return simulator (VRS) is a software package designed to simulate the venous hemodynamic parameters of the lower limbs. It is a virtual (digital) network of the lower extremity that computes in static and dynamic conditions the different variables (flow rate, venous diameter and internal pressure) for a defined external pressure.

It is a mathematical model solving the equations that govern the blood flow, applied to the venous network of the lower limbs.

For this purpose, a simplified model of the venous network is used. (figure 8)

The software takes into account the length and caliber of the veins, their distensibility, the blood viscosity, the valvular function (normal or pathological), the position and activity of the subject (standing, walking, lying) and the external pressure applied to the veins: muscular contraction, wearing of a medical compression stocking.

As a result, the software gives you the caliber, pressure and flow rate for any point of the virtual network.

The setting of the parameters was based on physiological data from the literature and the validation of the VRS software was published17 showing that the distal venous pressure at rest and during exercise are similar to the real pressures measured in vivo on healthy subjects and in different cases of valvular incompetence.

Hence, VRS could be considered as a reliable tool for simulation.

It has been used to assess the effect of the medical compression stockings on the venous return. A study has shown the impact of different levels of compression on (the) distal saphenous vein reflux. Another study was done to simulate the venous edema[19].

In the future, VRS could also be used to predict the hemodynamic consequences of venous surgery and other venous interventions. …

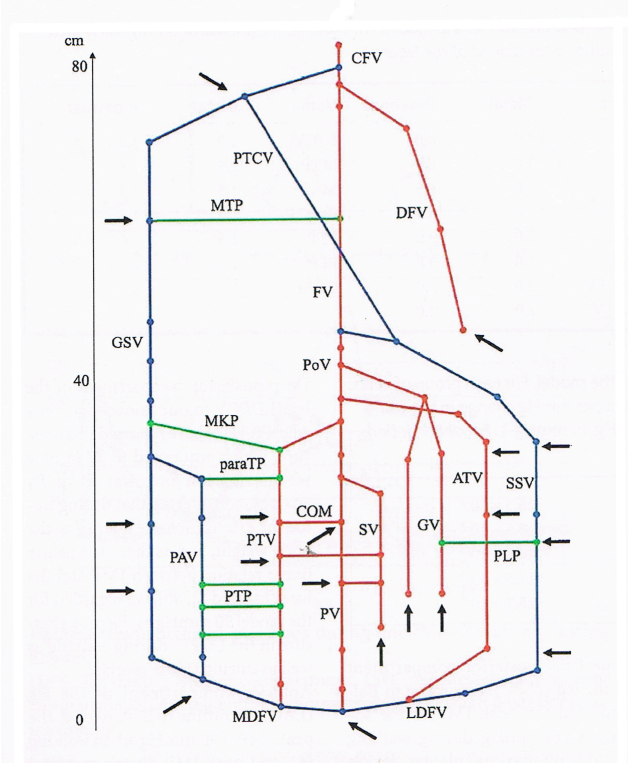

*Figure 8 - Two-dimensional representation of the lower limbs venous network used in the model of the **VRS simulation software®**. The deep veins are in red, the superficial in blue and the perforators in green. CFV=common femoral vein DFV=deep femoral vein PoV=popliteal vein G=Giacomini PTV=posterior tibial ATV=anterior tibial GV=gastrocnemius vein SV= soleus muscle MDFV=medial dorsal foot LDFV=lateral dorsal foot COM=perforators MTV=medial thigh perforator MKP=medial knee perforator paraTP=paratibial perforator PTP=tibial posterior perforator PLP=posterior leg perforator.*

3 - **CAAD (Computer Assisted Anatomical Dissection)**:

Classical anatomical study methods have limitations regarding the micro-anatomy of the location of vessels and nerves. The combination of immuno-histochemical methods and three-dimensional reconstruction could be used to resolve these limitations of morphological sciences.

The technique of computer assisted anatomical dissection (CAAD) is an original method applied in morphological science.

Originally created by Yucel[20,21] to describe the topography of the perineal nerves, the CAAD was first applied in our URDIA research unit by Karam et al. to study the distribution of the nerves of urethra[22,23] and intra-pelvic nerves[24,25], and then also for the whole reconstruction of young human embryo lower limbs[26,27].

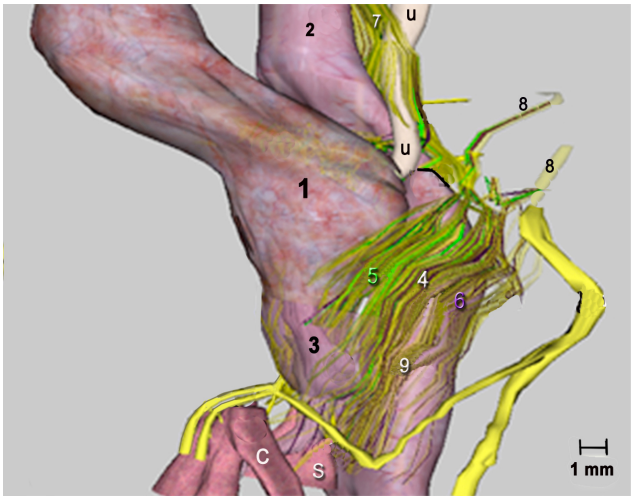

*Figure 9 - **Computer-assisted anatomic dissection** of 17-week-old male human fetal pelvis, Lateral view of the **inferior hypogastric plexus** (4), adrenergic fibers (5) in green and cholinergic fibers (6) in purple, hypogastric nerves (7) and pelvic splanchnic nerves (8) contain both fibers' types, adrenergic fibers mostly situated in the superior portion of the plexus, cholinergic fibers intend to concentrate in inferior portion. 1=bladder 2=Rectum 3=Prostate C=Corpus Cavernosum S= Corpus Spongiosum u=ureter, 9= Cavernous Nerve.*

Material and methods:

Serially transverse sections of the embryos' pelvic portion and lower limbs were serially immune-histochemically treated and digitized with high optical resolution scanner. The nerves were stained by Protein S100 immune marker and vessels by D240. The sections were aligned with Photoshop® software then a tridimensional reconstruction was achieved by manual segmentation of the anatomical structures using the WinSurf® software version 3.5.

Results: This produced a realistic 3D vectorial model of the pelvis and lower limb.

Regarding the pelvis, the three-dimensional reconstruction of serial histological sections stained by immune markers allowed identifying the precise structure and the innervations of the whole intra-pelvic organs with demonstration of the precise location of both adrenergic and cholinergic pathways[24,25]. A virtual dissection could be performed for each of the pelvic structures. We could describe the precise location of the inferior hypogastric plexus as well as the nature and the distribution of its fibers. (figure 9)

The evolution of CAAD confirmed and clarified topographic and surgical anatomy of the intra-pelvic innervations. This technique represents an original method in anatomical research and a great educational tool for surgeons in order to better understand the anatomy of an inaccessible region by conventional techniques.

Regarding the lower limbs, the 3D reconstruction of the whole limbs produces a virtual dissection of the muscles, nerves, vessels which is a precious tool to study the embryogenesis of the nervous and vascular system of the human embryo[26] (figure 10), especially of the venous network. In fact, today, there is no direct observation showing the different steps of the venous organogenesis of the human embryo.

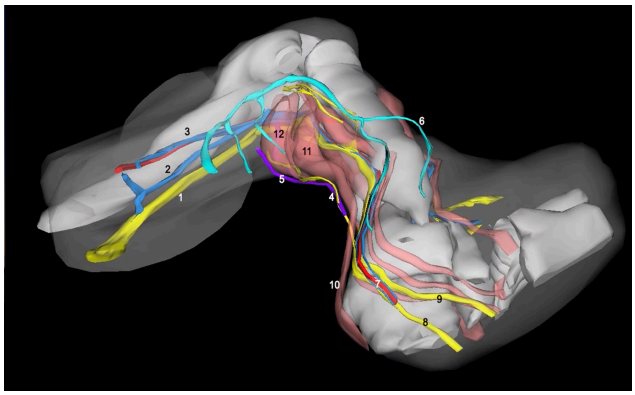

Figure 10 - Three-dimensional reconstruction of a 14 weeks old fetal left lower limb: 1 sciatic nerve, 2 axial vein, 3 femoral vein and artery, 4 sural nerve, 5 small saphenous vein, 6 great saphenous vein, 7 posterior tibial vessels, 8 lateral tibial nerve, 9 medial tibial nerve, 10 Achilles tendon, 11 soleus muscle, 12 medial gastrocnemius muscle.

4 - **3D modeling of the arterial system**:

The main use of *3D reconstruction of the arteries* since years is the investigation of 3D arterial reconstruction using CT angiography has traditionally been used for patients suffering peripheral arterial disease. Additional color duplex assessment may better evaluate arterial lesions by quantifying flow in areas of stenosis or dilatation, enabling better choice of treatment options.

More recently, a new field of application of 3D arterial modeling appeared with the onset of endovascular procedures and minimally invasive surgical techniques[28].

New algorithms were developed to improve the quality of the 3D reconstruction and shorten the computing time.

Software's providing functions of segmentation and simulation:

Mevislab is a medical Image Processing and Visualization modular framework downloadable for free at www.mevislab.de. It represents a powerful tool for image processing research and development with a special focus on medical imaging. It allows fast integration and testing of new algorithms and the development of clinical application prototypes.

Vascular modeling tool kit software package[30] (VMTK www.vmtk.org) is a collection of libraries and tools for 3D reconstruction, geometric analysis, mesh generation and surface data analysis for image-based modeling of blood vessels.

3D modeling is a kind of revolution in the management of vascular disease . It is a fantastic new tool which greatly assists the surgeon in surgical planning, simulation, training and augmented reality[29].

Surgical planning

By the use of dedicated simulation software, the preparation for an endovascular procedure is enhanced : it uses the patient's specific CT data the day before operation, and then performs a procedure rehearsal, which is reproducible and reliable.

Simulation and training of endovascular procedures is in fact a new paradigm for vascular trainees. Endovascular simulation improves the quantitative and qualitative performance of general surgery residents performing diagnostic angiography, in a randomized-controlled study[30] (Chaer al. 2006)

Endovascular Simulation has also proved its efficiency and competence in Thoracic Endo-Vascular Aortic Repair (TEVAR) Procedures[31]. Several options of simulation stations and tearing modules are proposed on the internet (endovascular knowledge blog at www.mentice.com)

Augmented reality[32] for minimally invasive and endoscopic surgery. The new technological advances in

optics, instrumentation, robotics, and computer systems provide the surgeon with new possibilities and capabilities : augmented reality technologies now provide guidance and navigation during surgical procedures. In addition, robotic devices[33] can enhance the surgeon's' capabilities in terms of dexterity and accuracy.

Finally, customization of vascular stents has been achieved, in particular for aorta. The customized aortic stent grafts are patient-specific in that they conform to the part of the ascending aorta, aortic arch and/or thoracic aorta. The 3D model of the vascular endoprothesis is based on a 3D image of the patient's anatomy. (patents filed in 2013 : EP2903561A1, US20150209162)

Conclusions

The 3dD modeling of vessels has recently brought us a revolution in the diagnosis and treatment of the vascular diseases:

For veins, the CT venography is a new mapping tool in addition to color duplex ultrasound.

For arteries, it is a true revolution for vascular & endovascular treatments, for surgical planning, training and augmented reality techniques.

It is also a remarkable learning tool for the whole of human anatomy.

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Journal of Theoretical and Applied Vascular Research
The Official Journal of the Vasculab Foundation

Volume 1 Jul 2016 Issue 1

JTAVR
A Journal on Research in Vascular Diseases

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