

Submission ID #: 70986

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Title: A Comprehensive Rat Model for Assessing Hepatic Transport Pathways Through Simultaneous Lymphatic and Blood Vascular Sampling

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

If **Yes**, can you record movies/images using your own microscope camera?

No

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

Zeiss AxioCam ERc5s and Stemi 2000-CS

2.2.1 Horizontal skin incision

2.2.2 Skin retraction and muscle incisions

2.4.1 Hepatic lymph duct adjacent to hepatic artery and vena cava

2.4.2 Mesenteric lymph duct (opaque/white)

2.4.3 Hepatic lymph duct (translucent/narrow)

2.5.1 Removal of connective tissue over lymph duct and vena cava

2.5.2 Forceps insertion beneath right kidney fat bed

2.5.3 Forceps tips emerging beneath vena cava

2.5.5 Cannula pulled through tunnel

2.6.2 Puncturing hepatic lymph duct

2.7.2 Cannula insertion into lymph duct

2.7.3 Lymph flow confirmation at cannula

2.8.1 Application of veterinary adhesive

2.8.2 Flow through tubing after adhesive

2.8.3 Removal of gauze

2.8.4 Organs repositioned

2.9.2 Lymph drop-wise collection

4.1.1 Reopening abdominal cavity

4.1.2 Liver retraction

4.2.1 Identification of hepatic artery

***Videographer:** Please film the abovementioned shots using a scope kit.*

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **Yes.**

If **Yes**, how far apart are the locations? **Same building.**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 16

Number of Shots: 51

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Videographer's NOTE:

I have begun uploading the files from today's (April 30) shoot 70986. The scope attachment and phone filming was done in 4K on my iphone to ensure we had good enough quality through the eyepiece. The camera files are a total of 19Gbs for the XDROOT clips and headshots. Iphone files are about 12Gb's and will be uploaded in their own folder once the other files have made it across.

Here are my on set notes and one BTS photo. The iphone and microscope was not practical to use the slate under the lens or remove and replace on the device so I had a verbal slate on the files and marked them as such. There were a few takes that the team on site thought it would make more sense to have either under scope or on camera instead of what was listed, so I recorded on both and tried to sync when I could. The end of the shoot also had the artery nicked so we faked and only had a small amount of blood drawn to put into the capsule/test tube.

INTRODUCTION:

REQUIRED: Summarize your research focus and the main questions it aims to answer.

- 1.1. **Natalie L Trevaskis:** Our lab studies the role of the lymphatic system in inflammatory and cardiometabolic diseases and develops targeted lymphatic drug delivery strategies to treat them.

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Peng Li:** This protocol enables evaluation of hepatic processes including drug metabolism, nutrient flux, immune signaling, and liver responses under diverse conditions.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Andrea Chan:** This protocol enables simultaneous measurement of hepatic lymph and blood to evaluate transport, metabolism, and clearance of molecules including drugs, nutrients, and signals.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Andrea Chan:** Precise cannulation of the hepatic lymph duct is technically challenging and requires careful anatomical identification and surgical technique.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Sanjeevini Babu Reddiar:** Future studies can use this technique to map hepatic transport, clearance, and metabolism, advancing understanding of liver diseases and targeted therapies.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

1.6 **Natalie L Trevaskis, Professor, Monash Institute of Pharmaceutical Sciences:** (authors will present their testimonial statements live)

1.6.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What is the key takeaway you hope viewers remember after watching your video article?

1.7 **Sanjeevini Babu Reddiar, Senior Research Fellow, Monash Institute of Pharmaceutical Sciences:** (authors will present their testimonial statements live)

1.7.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Monash Institute of Pharmaceutical Sciences
Animal Ethics Committee

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Surgical Exposure and Hepatic Lymph Duct Identification in Rats

Demonstrator: Andrea Chan

- 2.1. To begin, obtain a male Sprague–Dawley rat weighing around 250 to 300 grams, successfully cannulated via carotid artery and jugular vein, ready for surgery [1]. Position the anesthetized rat in dorsal recumbency under a surgical microscope [2-TXT].
 - 2.1.1. WIDE: Show the surgical setup and zoom in to show the cannulated rat ready for the upcoming procedure.
 - 2.1.2. Talent positioning the rat in dorsal recumbency under the surgical microscope.
TXT: Anesthesia: 1–3% isoflurane
- 2.2. Make a 4-to-5-centimeter horizontal skin incision from the midline to the right flank, approximately 1.5 to 2 centimeters below the ribcage [1]. Retract the skin and make matching incisions in the external and internal oblique muscles [2]. **NOTE: This is audio labelled**
 - 2.2.1. SCOPE: Shot of a horizontal skin incision being made from the midline to the right flank below the ribcage.
 - 2.2.2. SCOPE: Shot of the skin being retracted and matching incisions being made in the external and internal oblique muscles.
- 2.3. Cauterize or ligate any large blood vessels encountered during the procedure [1]. Use saline-moistened cotton swabs to move the liver toward the diaphragm [2], the stomach to the left [3], and the small intestine inferiorly [4]. Keep the organs retracted with saline-soaked gauze [5]. **NOTE: This step is filmed in a single shot**
 - 2.3.1. Talent cauterizing or ligating a large blood vessel in the surgical field.
 - 2.3.2. Talent moving the liver toward the diaphragm with saline-moistened cotton swabs.
 - 2.3.3. Talent moving the stomach to the left with saline-moistened cotton swabs.
 - 2.3.4. Talent moving the small intestine inferiorly with saline-moistened cotton swabs.
 - 2.3.5. Talent placing saline-soaked gauze to maintain organ retraction.

- 2.4. Identify the hepatic lymph duct running parallel to and slightly caudal to the hepatic artery, medial to the right kidney, and adjacent to the inferior vena cava [1]. Use the larger opaque or white mesenteric lymph duct [2] as an anatomical landmark to identify the translucent and narrow hepatic lymph duct located caudally [3]. Use straight Graefe forceps to remove the connective tissue overlying the lymph duct and vena cava [1].

NOTE: This step is filmed in a single shot and is audio labelled

- 2.4.1. SCOPE: Shot of the hepatic lymph duct running parallel to the hepatic artery and adjacent to the inferior vena cava.
- 2.4.2. SCOPE: Shot of the opaque or white mesenteric lymph duct.
- 2.4.3. SCOPE: Shot of the translucent and narrow hepatic lymph duct.
- 2.4.4. SCOPE: Show the connective tissue overlying the lymph duct and vena cava being removed with straight Graefe forceps.
- 2.5. Insert the forceps through the fat bed beneath the right kidney to create a tunnel under the vena cava [1]. Advance the forceps until the tips emerge parallel to the hepatic lymph duct on the opposite side of the vena cava [2]. Use the forceps to grasp the external end of the pre-prepared cannula [3] and pull the cannula through the tunnel until its tip emerges on the opposite side [4].
- 2.5.1. SCOPE: Show the straight Graefe forceps being inserted through the fat bed beneath the right kidney.
- 2.5.2. SCOPE: Shot of the forceps tips emerging beneath the vena cava parallel to the hepatic lymph duct.
- 2.5.3. Talent gripping the external end of the pre-prepared cannula with straight Graefe forceps.
- 2.5.4. SCOPE: Show the cannula being pulled through the tunnel beneath the vena cava until the tip emerges on the opposite side.

3. Hepatic Lymph Cannulation and Post-Surgical Sample Collection

Demonstrator: Andrea Chan

- 3.1. Use the Halsted-Mosquito hemostat forceps to bend the tip of a 25-gauge needle to approximately 90 degrees [1]. Using the bent needle, carefully puncture the hepatic lymph duct on the right side closest to the operator [2].
- 3.1.1. Talent bending the tip of a 25-gauge needle with the Halsted-Mosquito hemostat forceps.
- 3.1.2. SCOPE: Show the hepatic lymph duct on the right side being punctured with the bent needle.

- 3.2. Check the cannula pre-filled with anticoagulant solution and remove air bubbles, if any [1]. Insert the bevelled end approximately 2 millimeters into the duct [2]. Confirm lymph flow at the external end of the cannula at approximately 100 microliters per hour [3]. Adjust or reinsert the cannula if no flow is observed [4]. **NOTE: Match scope and camera**
- 3.2.1. Talent checking the cannula filled with anticoagulant solution for air bubbles and removing any air bubbles if present.
 - 3.2.2. SCOPE: Talent inserting the bevelled end of the cannula approximately 2 millimeters into the duct.
 - 3.2.3. Shot of lymph flow at the external end of the cannula.
 - 3.2.4. SCOPE: Talent adjusting or reinserting the cannula.
- 3.3. Plug the cannula at the collecting end, then apply a small drop of veterinary adhesive to secure the insertion site [1]. Once the adhesive is dry, confirm lymph flow through the tubing [2]. Afterward, remove the gauze [3] and return the organs to their original positions [4].
- 3.3.1. SCOPE: Shot of a small drop of veterinary adhesive being applied to the insertion site. **NOTE: This is audio labelled**
 - 3.3.2. Shot of lymph flowing through the tubing after the adhesive has dried.
 - 3.3.3. SCOPE: Shot of the gauze being removed from the surgical field. **NOTE: 3.3.3 and 3.3.4 are audio labelled**
 - 3.3.4. SCOPE: Shot of the organs being returned to their original positions.
- 3.4. Place the collection tube containing anticoagulant solution below the cannula outlet [1]. Confirm that lymph is flowing drop-wise into the tube [2]
- 3.4.1. Talent placing the collection tube containing anticoagulant solution below the cannula outlet. **NOTE: 3.4.1 and 3.4.2 are filmed in a single shot**
 - 3.4.2. Shot of lymph flowing drop-wise into the collection tube.

~~4. Post-Surgical Lymph and Blood Collection~~ **NOTE: This section is merged with the previous section**

~~Demonstrator: Peng Li~~

- 4.1. Next, start sterile saline infusion at 1.5 to 3.0 milliliters per hour through the jugular vein cannula immediately after cannulation [1]. Monitor breathing and reflexes while maintaining anesthesia via a nose cone [2-TXT].
- 4.1.1. Talent initiating sterile saline infusion through the jugular vein cannula.
 - 4.1.2. Talent monitoring the breathing patterns and reflex responses.
TXT: Anesthesia via nose cone: 1-3% isoflurane in oxygen (0.4-0.6 L/min)

4.2. After therapeutic dosing via the jugular vein cannula, collect hepatic lymph continuously into tubes containing anticoagulant solution [1]. Replace the collection tubes hourly or as needed [2]. Collect arterial blood samples at designated time points, keeping total volume below 10 percent of estimated circulating blood volume before terminal sampling [3]. **NOTE: This step is filmed in a single shot**

- 4.2.1. Talent collecting hepatic lymph into tubes containing anticoagulant solution.
- 4.2.2. Shot of the talent replacing a filled collection tube with a fresh one.
- 4.2.3. Shot of the talent collecting an arterial blood sample at a designated time point.

5. Hepatic Artery Cannulation and Blood Collection

Demonstrator: Andrea Chan

5.1. Reopen the abdominal incision to expose the upper abdominal cavity while maintaining deep anesthesia and stable respiration [1]. Use saline-moistened gauze to gently retract the liver toward the diaphragm, the stomach to the left, and the small intestine inferiorly [2].

- 5.1.1. SCOPE: Show the abdominal incision being reopened to expose the upper abdominal cavity. **NOTE: SCOPE/Camera, this is audio labelled**
- 5.1.2. SCOPE: Show the liver being retracted toward the diaphragm, stomach to the left, and small intestine inferiorly with saline-moistened gauze.

5.2. Identify the hepatic artery running parallel to the hepatic lymph duct, situated adjacent to the mesenteric lymph duct [1]. Obtain a polyvinyl cannula with an outer diameter of 0.61 millimeters and an inner diameter of 0.28 millimeters, prefilled with an anticoagulant solution, and remove any air bubbles [2].

- 5.2.1. SCOPE: Visualize and confirm the hepatic artery in the surgical field. **NOTE: this is audio labelled**

Videographer: Please hold focus on the hepatic artery for a few seconds to allow the pulsation to be clearly observable.

- 5.2.2. Talent checking the cannula filled with anticoagulant solution for air bubbles and removing any air bubbles if present.

5.3. Using curved iris forceps, isolate a short segment of the hepatic artery [1]. Place a 4-0 silk suture beneath the artery [2]. Gently lift the artery and place a second suture distal to the first [3]. Tie the distal suture to control backflow while leaving the proximal suture untied for later closure [4]. Using fine-point microscissors, make a small transverse incision approximately 0.5 millimeters in the artery wall between the sutures [5].

- 5.3.1. Talent isolating a short segment of the hepatic artery with curved iris forceps.
NOTE: 5.3.1-5.3.4 are filmed in a single shot
- 5.3.2. Talent placing a 4-0 silk suture beneath the hepatic artery.
- 5.3.3. Talent lifting the artery and placing a second suture distal to the first.
- 5.3.4. Talent tying the distal suture while leaving the proximal suture untied.
- 5.3.5. SCOPE: Talent making a small transverse incision in the artery wall with fine-point micro-scissors. **NOTE: 5.3.5-5.4.4 are filmed in a single shot and are audio labelled**

- 5.4. Use the tip of the Dumont forceps to lift the vessel flap to stabilize the vessel lumen for cannula insertion [1]. Insert the bevelled end of the polyvinyl cannula into the opening [2] and advance the cannula approximately 2 to 3 millimeters [3]. Secure the cannula by tying the proximal suture around both the vessel and cannula [4].
 - 5.4.1. SCOPE: Talent lifting the vessel flap with the tip of the Dumont forceps.
 - 5.4.2. SCOPE: Talent inserting the bevelled end of the polyvinyl cannula into the arterial opening.
 - 5.4.3. SCOPE: Talent advancing the cannula approximately 2 to 3 millimeters.
 - 5.4.4. SCOPE: Talent tying the proximal suture around the vessel and cannula.

- 5.5. Confirm correct placement by gently aspirating a small amount of bright red arterial blood [1]. Collect 0.5 to 1 milliliter of blood into a pre-labelled tube containing anticoagulant [2]. After collection, withdraw the cannula [3] and tighten both sutures [4].
 - 5.5.1. Talent gently aspirating a small amount of bright red arterial blood. **NOTE: 5.5.1-5.5.4 are filmed in a single shot**
 - 5.5.2. Talent collecting blood into a pre-labelled tube containing anticoagulant.
 - 5.5.3. Talent withdrawing the cannula after blood collection.
 - 5.5.4. Talent tightening both sutures to achieve hemostasis.

Results

6. Results

6.1. Lymphatic recovery of intravenously administered macromolecular therapeutics across thoracic, hepatic, and mesenteric lymph demonstrates the model's utility for evaluating lymphatic transport [1].

6.1.1. LAB MEDIA: Figure 8. *Video editor: Show the full figure and emphasize the label and graph for thoracic, hepatic, and mesenteric in sequence to align with the narration.*

6.2. Hepatic lymph exhibits a markedly lower total lipid content compared with thoracic and mesenteric lymph [1].

6.2.1. LAB MEDIA: Figure 9. *Video editor: Highlight the yellow portion of the pie chart for hepatic lymph showing 8 percent*

6.3. Protein concentrations in hepatic lymph are comparable to those of thoracic and mesenteric lymph [1].

6.3.1. LAB MEDIA: Figure 9. *Video editor: Highlight the blue portions of all three pie charts showing similar large protein segments in mesenteric, hepatic, and thoracic lymph*

1. Sprague–Dawley

Pronunciation link: <https://www.merriam-webster.com/dictionary/Sprague-Dawley>

IPA: /ˌsprāg-ˈdō-lē/

Phonetic Spelling: sprayg·daw·lee

2. Cannulated

Pronunciation link: <https://www.merriam-webster.com/dictionary/cannulated>

IPA: /ˈkən-yə-ˌlā-təd/

Phonetic Spelling: kan·yuh·lay·tuhd

3. Carotid

Pronunciation link: <https://www.merriam-webster.com/dictionary/carotid>

IPA: /kəˈrɑːtɪd/

Phonetic Spelling: kuh·raa·tuhd

4. Jugular

Pronunciation link: <https://www.merriam-webster.com/dictionary/jugular>

IPA: /ˈdʒʌɡjələr/

Phonetic Spelling: juh-g·yuh·ler

5. Dorsal

Pronunciation link: <https://www.merriam-webster.com/dictionary/dorsal>

IPA: /ˈdɔːrsəl/

Phonetic Spelling: dor·suhl

6. Recumbency

Pronunciation link: <https://www.merriam-webster.com/dictionary/recumbency>

IPA: /rɪˈkʌmbənsi/

Phonetic Spelling: ruh·kuhm·buhn·see

7. Oblique

Pronunciation link: <https://www.merriam-webster.com/dictionary/oblique>

IPA: /əˈbliːk/

Phonetic Spelling: uh·bleek

8. Cauterize

Pronunciation link: <https://www.merriam-webster.com/dictionary/cauterize>

IPA: /ˈkɔːtəˌraɪz/

Phonetic Spelling: kaw·tuh·ryze

9. Diaphragm

Pronunciation link: <https://www.merriam-webster.com/dictionary/diaphragm>

IPA: /ˈdaɪəˌfræm/

Phonetic Spelling: dye·uh·fram

10. Inferiorly

Pronunciation link: <https://www.merriam-webster.com/dictionary/inferiorly>

IPA: /ɪnˈfɪəriəli/

Phonetic Spelling: in·feer·ee·er·lee

11. Hepatic

Pronunciation link: <https://www.merriam-webster.com/dictionary/hepatic>

IPA: /hɪˈpætɪk/

Phonetic Spelling: huh·pat·ik

12. Mesenteric

Pronunciation link: <https://www.merriam-webster.com/dictionary/mesenteric>

IPA: /ˌmezənˈterɪk/

Phonetic Spelling: mez·uhn·ter·ik

13. Translucent

Pronunciation link: <https://www.merriam-webster.com/dictionary/translucent>

IPA: /trænzˈluːsənt/

Phonetic Spelling: tranz·loo·suhnt

14. Caudally
Pronunciation link: <https://www.merriam-webster.com/dictionary/caudal>
IPA: /'kɔːdəl/
Phonetic Spelling: kaw·duhl·lee
15. Graefe
Pronunciation link: <https://www.howtopronounce.com/graefe>
IPA: /'greɪfə/
Phonetic Spelling: gray·fuh
16. Vena cava
Pronunciation link: <https://www.merriam-webster.com/dictionary/vena%20cava>
IPA: /ˌviːnə 'keɪvə/
Phonetic Spelling: vee·nuh kay·vuh
17. Cannula
Pronunciation link: <https://www.merriam-webster.com/dictionary/cannula>
IPA: /'kænjələ/
Phonetic Spelling: kan·yuh·luh
18. Halsted-Mosquito
Pronunciation link: <https://www.howtopronounce.com/halsted>
IPA: /'hɔːlstəd/
Phonetic Spelling: hawl·sted
19. Hemostat
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemostat>
IPA: /'hiːmə stæt/
Phonetic Spelling: hee·muh·stat
20. Bevelled
Pronunciation link: <https://www.merriam-webster.com/dictionary/beveled>
IPA: /'bevəld/
Phonetic Spelling: bev·uhld
21. Anticoagulant
Pronunciation link: <https://www.merriam-webster.com/dictionary/anticoagulant>
IPA: /ˌæntiˌkoʊ'æɡjələnt/
Phonetic Spelling: an·tee·koh·ag·yuh·luhnt
22. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /'maɪkrəˌliːtər/
Phonetic Spelling: my·kruh·lee·ter
23. Veterinary
Pronunciation link: <https://www.merriam-webster.com/dictionary/veterinary>
IPA: /'vet(ə)rəˌneri/
Phonetic Spelling: vet·ruh·nair·ee
24. Isoflurane
Pronunciation link: <https://www.merriam-webster.com/medical/isoflurane>

IPA: /ˌaɪsoʊˈflɔːrɪn/

Phonetic Spelling: eye·soh·floor·ayn

25. Therapeutic

Pronunciation link: <https://www.merriam-webster.com/dictionary/therapeutic>

IPA: /ˌθerəˈpjʊːtɪk/

Phonetic Spelling: thair·uh·pyoo·tik

26. Polyvinyl

Pronunciation link: <https://www.merriam-webster.com/dictionary/polyvinyl>

IPA: /ˌpɑːliˈvaɪnəl/

Phonetic Spelling: pah·lee·vy·nul

27. Dumont

Pronunciation link: <https://www.howtopronounce.com/dumont>

IPA: /duːˈmɒnt/

Phonetic Spelling: doo·mont

28. Aspirating

Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirating>

IPA: /ˈæspəˌreɪtɪŋ/

Phonetic Spelling: ass·puh·ray·ting

29. Hemostasis

Pronunciation link: <https://www.merriam-webster.com/dictionary/hemostasis>

IPA: /ˌhiːməˈsteɪsɪs/

Phonetic Spelling: hee·muh·stay·suhs

30. Macromolecular

Pronunciation link: <https://www.merriam-webster.com/dictionary/macromolecular>

IPA: /ˌmækroʊməˈleɪkjələr/

Phonetic Spelling: mak·roh·muh·lek·yuh·ler

31. Thoracic

Pronunciation link: <https://www.merriam-webster.com/dictionary/thoracic>

IPA: /θəˈræɪk/

Phonetic Spelling: thuh·ras·ik

32. Lymphatic

Pronunciation link: <https://www.merriam-webster.com/dictionary/lymphatic>

IPA: /lɪmˈfætɪk/

Phonetic Spelling: lim·fat·ik

33. Intravenously

Pronunciation link: <https://www.merriam-webster.com/dictionary/intravenous>

IPA: /ˌɪntrəˈviːnəs/

Phonetic Spelling: in·truh·vee·nuhs·lee

34. Mesenteric

Pronunciation link: <https://www.merriam-webster.com/dictionary/mesenteric>

IPA: /ˌmezənˈterɪk/

Phonetic Spelling: mez·uhn·ter·ik

35.

