

Isolated Tissue Preparations

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Types

In vitro

Ex vivo

In vivo

In situ

In silico

Advantages

Several preparations from single animal

Small amount of drug

Drug effect is tested directly

Guinea pigs

Rabbits

Sources

Rats & Mice

Cats & Dogs

Isolated strips of intestine

Abundant material, more resistant & easy to set up

Variable spontaneous activity of different sections

Different type of pharmacological actions can be studied

Muscle tone

Biophase

Contact period of the Drug with the tissue

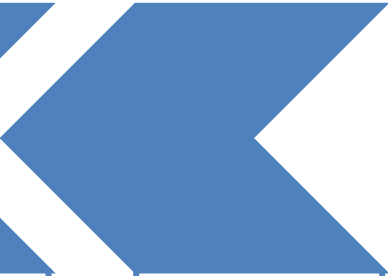
Rate of action of a Drug

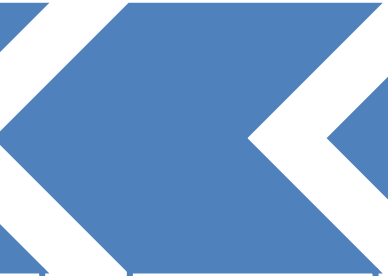
Process

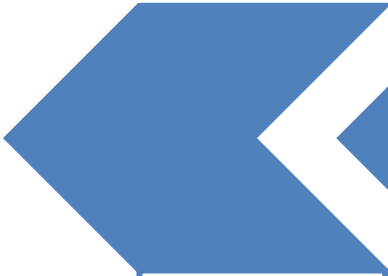
- Complete adjustment of organ bath
- Adjustment of lever

- Animal and tissue selection
- Surgical process and collection of a tissue

- Tissue attachment to organ bath
- Relaxation time to tissue

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- Prepare the standard drug
 - Test any concentration of drug for response

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- Standardize tissue response with same dose

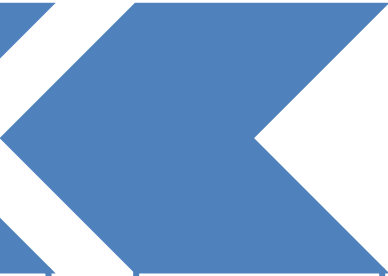
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- Prepare DRC for standard drug
 - Prepare DRC with test drug

Important Points to Remember

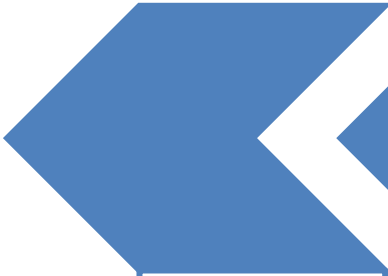
- Check Organ bath and PSS
- Writing Lever

- Dissection
- Rest

- Empty organ bath regularly
- Add drug slowly

- 
- Allow drug to act
 - Wash

- 
- Put a mark on drum

- 
- Use stopwatch during experiment
 - Start with a very low concentration

Physiological Salt Solution

- Also called as **PSS** / Ringer solution
- Maintains tissue outside the animal body
- Select PSS in which tissue last longer
- Prepare the solution with the help of distilled or double distilled or deionised water
- Prepare fresh solution

SOLUTION

USE

Tyrode solution

Mammalian smooth
muscle

Frog-Ringer solution

Amphibian tissue

Ringer-Locke solution

Heart muscle preparation

Krebs solution

Any tissue

- McEwen solution - sucrose in addition to glucose
- Krebs solution - used for any tissue
- DeJalon , Frog-Ringer and Ringer-Locke do not contain magnesium and phosphate
- Krebs, DeJalon and McEwen – aerated with carbogen and used for the mammalian isolated organ and avian skeletal muscle

Fast Contracting Smooth Muscle Preparation

TISSUE	DRUG
Guinea pig ileum	Histamine
Rat colon	Acetylcholine (ACh)
Rat uterus	ACh
Rat anococcygeus	ACh
Rat vas deferens	ACh
Guinea pig atria	Adrenaline (Adr)

Guinea Pig Ileum

- **AIM** :- To determine unknown concentration of **histamine** by using guinea pig ileum
- Guinea pig ileum is most sensitive to histamine .
- It has the spontaneous activity and specificity is improved by using atropine (for histamine assay) or mepyramine (for acetylcholine) in Tyrode.

- Ileum is preferred because of less mesentery attached to it, and nearly all receptors are present.
- 10 cm of ileum attached to the cecum should be excluded.
- Spontaneous activity of the tissue is reduced by performing the experiment 5-7°C lower than body temperature.

Rat Colon

- **AIM:-** To determine unknown concentration of **ACh** using rat ascending/descending colon.
- Sensitivity may increase by keeping colon at 4°C for 24 hr.
- Expression of calcium-sensing receptors(CaSR) mediates increase in inositol 1,4,5-trisphosphate.

- Easy to isolate and more handling resistant.
- For ACh , most sensitive tissue is dorsal leech muscle and frog rectus abdominis.
- Initial few centimeters of colon usually used for bioassay of Adr and NA.

Guinea Pig Atria

- **AIM:-** To determine unknown concentration of **Adr** using guinea pig atria
- Beta 2 receptors widely present in atria
- Required less trimming or slicing of the tissue
- **Advantages :-** Tissue thickness , easy separation of right and left atria , good contractile tension , stability etc

Rat Uterus

- AIM :-To determine unknown concentration of **ACh** using rat uterus
- Induce the estrus cycle in female rat
- Treat with stilbestrol 0.1mg/kg, SC ,
24 hrs before the experiment
- Other drugs like adrenaline , histamine,
oxytocin also sensitive to uterus

- Response depends on the animal age due to variation in estrus cycle .
- Other drugs like 5-HT,NA can be assayed .

Rat Vas Deferens

- **AIM** :- To determine unknown concentration of **ACh** using rat vas deferens
- Described by the Henderson et al(1972) and Hart et al(1979)
- Rat and guinea pig are suitable animals
- Animal should be fed with oats for 3 days .

- Adrenaline and phenylephrine contract the vas deferens whose action is blocked by the phentolamine .

Rat Anococcygeus

- **AIM:** To determine unknown concentration of **ACh** using rat anococcygeus muscle preparation
- Described by Gillespie (1972)
- It arises from sacral vertebrae and reaches to terminal colon (near anus).
- It has tendinous origin and do not appear soft.

- It has a dense adrenergic excitatory and inhibitory innervations .
- Insensitive to histamine .

Slow Contracting Muscle

TISSUE	DRUG
Rat fundus	Serotonin (5-HT)
Frog rectus abdominis	ACh
Guinea pig trachea	ACh
Rat phrenic nerve diaphragm	ACh
Chick biventral cervicis	Neuromuscular blocking agents

Rat Fundus

- **AIM** :-To determine unknown concentration of **5-HT** using rat stomach (fundus)
- Stomach fundus is most sensitive tissue among the whole parts of stomach.
- fundus identification – gray color , above the pink thick pylorus.
- Insensitive to histamine

- Animal should be fasted properly.
- Fundus contains the swallowed air.
- Sometimes it needs a stretching weight.
- Both longitudinal and circular muscles may be used in the experiment which depends on the transverse cut made to prepare the tissue.

Frog Rectus Abdominis

- **AIM** :- To determine unknown concentration of **ACh** using frog rectus abdominis muscle.
- It's a striated skeletal muscle sensitive to ACh and the easiest isolated tissue to handle.
- Contains more of multiple-innervated fibers so show the slow contraction.
- Frog, being an amphibian animal, it responds under room temperature.

Guinea Pig Trachea

- **AIM** :- to determine unknown concentration of **ACh** using guinea pig trachea
- Extraction of at least 6 cm of trachea required
- Tracheal ring is in “D” form and smooth muscle is present in the straight line of “D” shape.
- Response is slow to develop but last for longer period

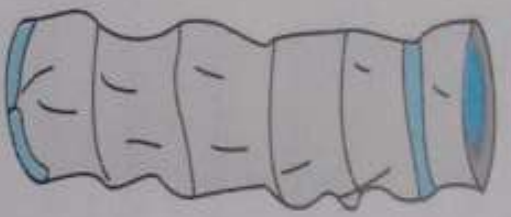
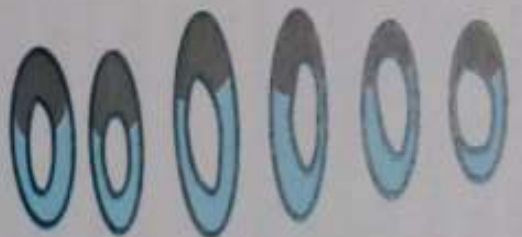
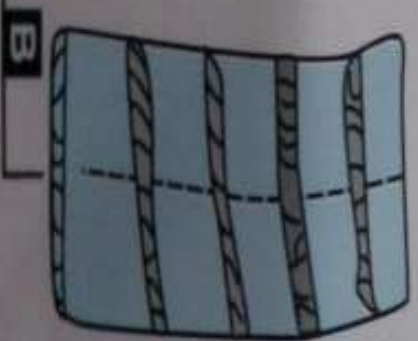
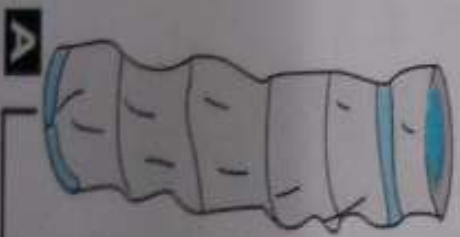
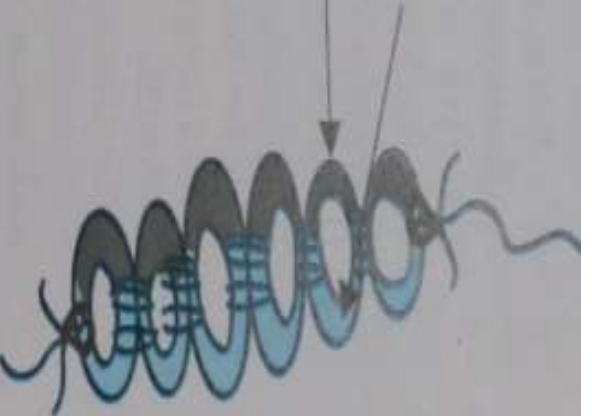


Fig. 16.3: Separation of tracheal rings and method to make a chain



Cartilage
Smooth muscle



Figs 16.4A to C: Another method to prepare trachea for bioassay

- Mainly to demonstrate the respiratory dominant adrenoceptor so utilized to study the bronchodilators like Adr , theophylline.
- Ideal model to study the contractile drugs like Ach,5-HT and histamine, and their antagonism can be studied by drugs like Adr , aminophylline.

Rat Phrenic Nerve Diaphragm

- **AIM** :- To determine unknown concentration of **ACh** using rat phrenic nerve diaphragm
- This is primary motor nerve of the diaphragm which arises mainly from the 4th cervical nerve.
- Useful for evaluation for neuromuscular function

Chick Biventer-Cervicis

- **AIM** : - To determine neuromuscular blocking drugs using innervated biventer-cervicis preparation
- Biventer-cervicis – an anatomically complex tendinous muscle
- Responds differently twitch / contraction to different stimuli

Cardiac Muscle Preparation

**Effect of various drugs on Isolated heart
(Langendorff's preparation)**

**Effect of different drugs on Normal and
Hypodynamic Rabbit heart**

**Effect of inotropic and chronotropic effects of
various drugs on Frogs heart**

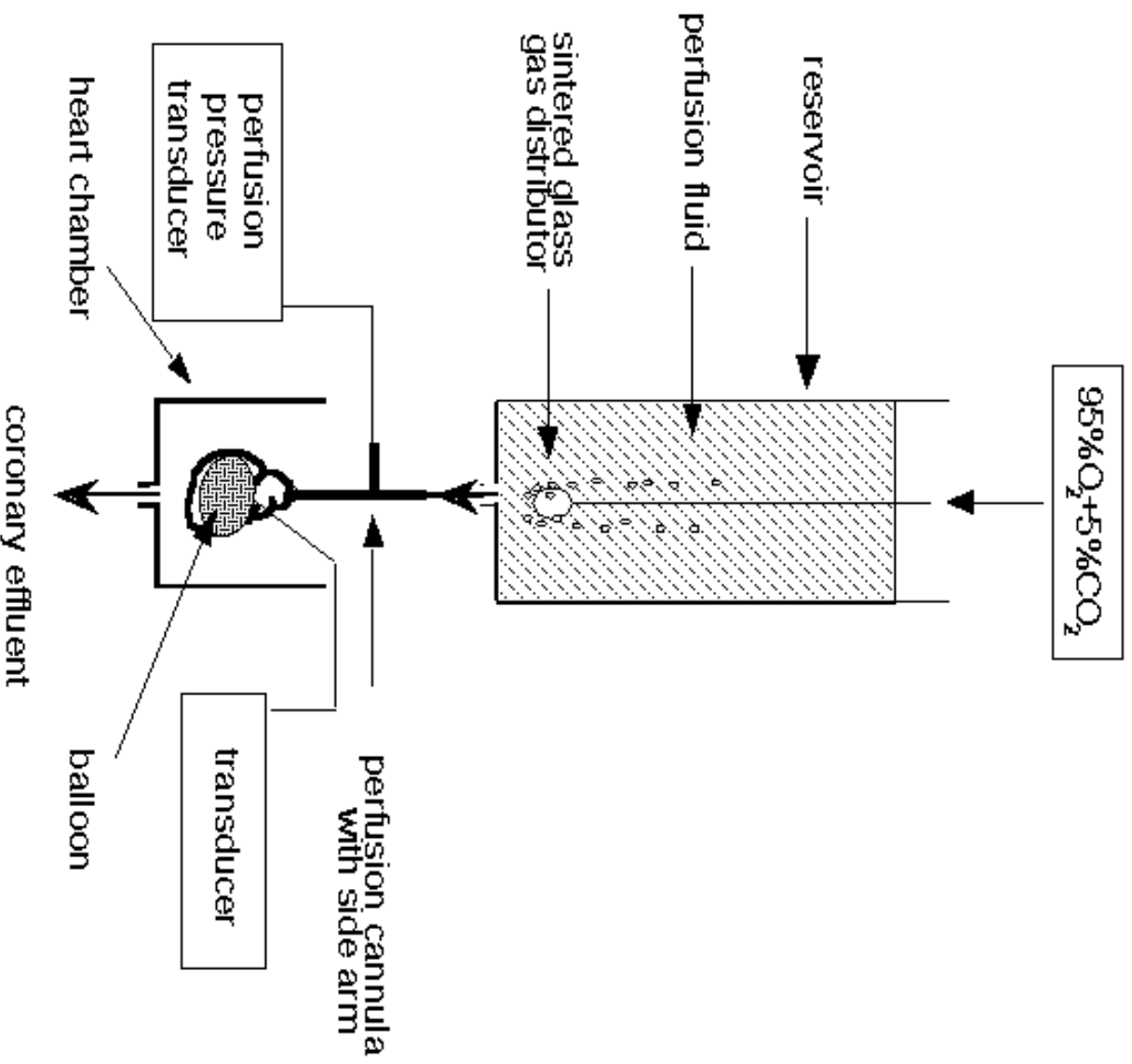
Langendorff's Preparation

- **AIM** :- To observe the effect of various drugs on Isolated heart
- In 1897, **Oscar Langendorff** established the isolated mammalian heart preparation.
- Based on principle of retrograde flow in aorta either at constant flow or constant pressure .

- **Animals :-**
 - Albino Rats**
 - New Zealand Rabbits**
 - Guinea pig**
 - **23° ± 2° C temperature , 12:12 hr light and dark cycle ,free access to tap water and food**
ad libitum

- Animal is pretreated with the heparin .
- The recording is done by attaching the thread to a **strain gauge transducer** , an attachment with the student physiograph

Figure 1



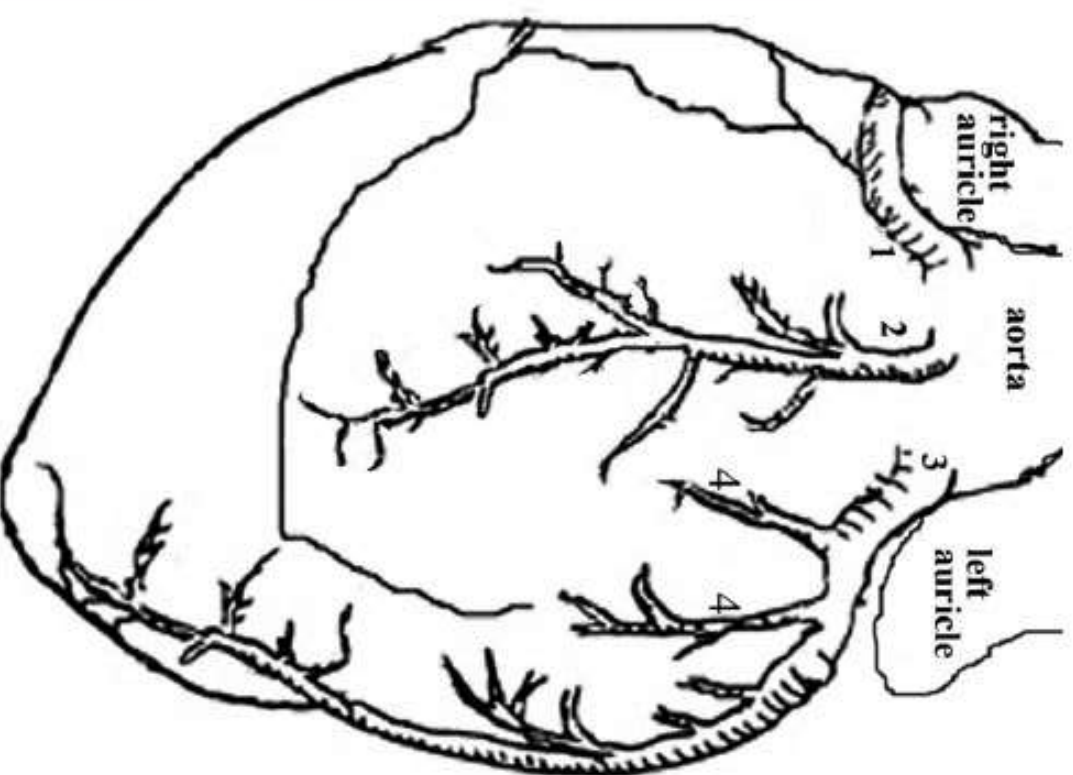
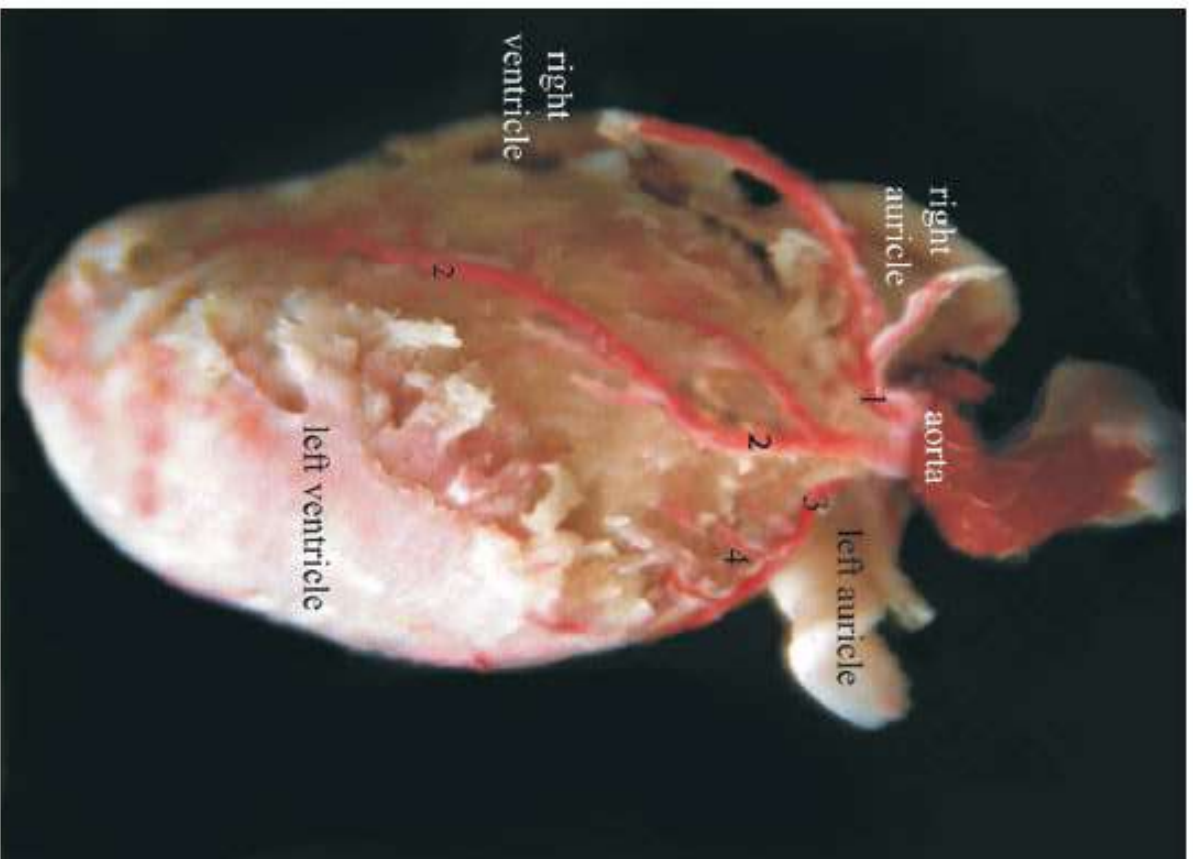
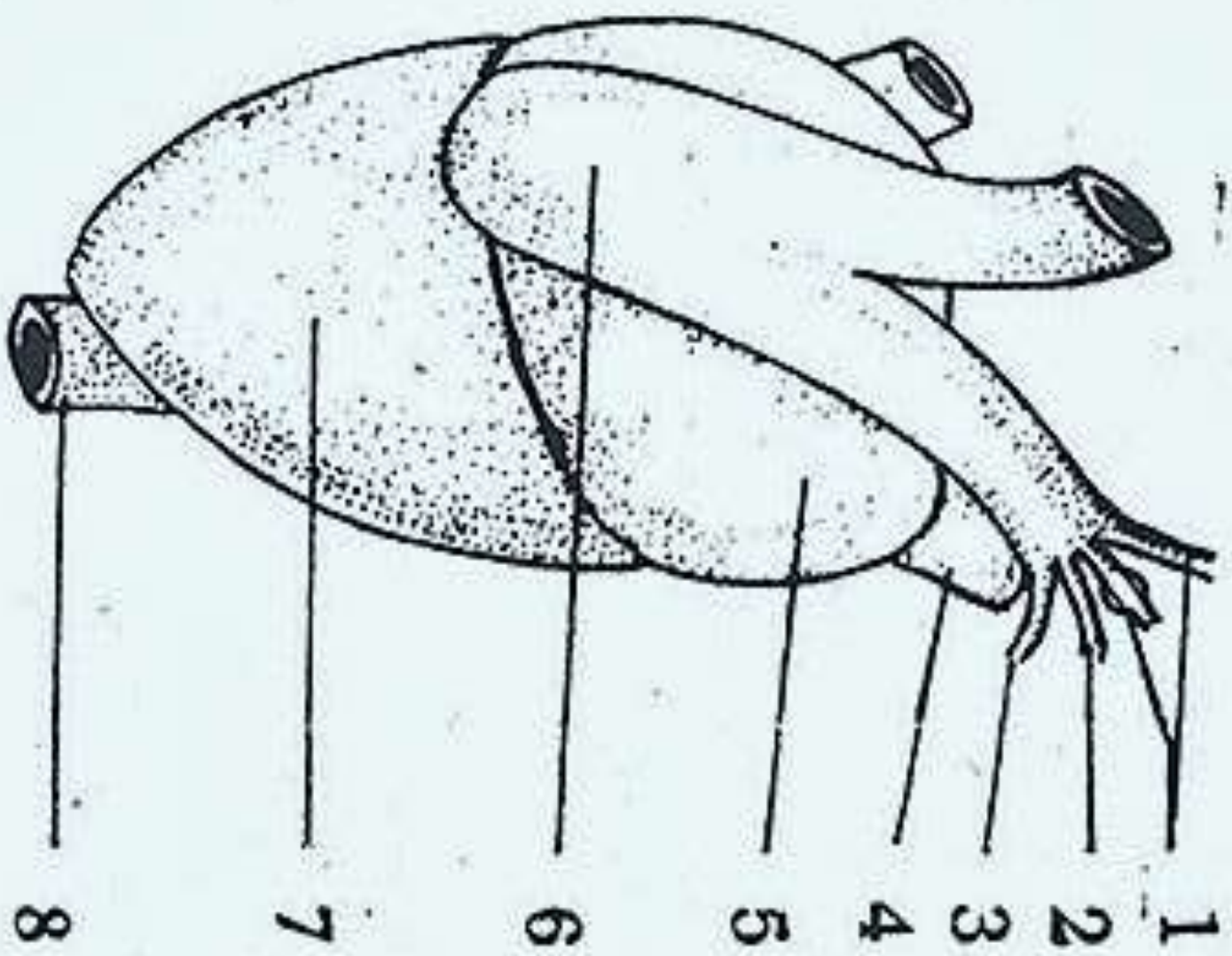


Fig. 4. A, **Different origin point of the ramus septalis**; B, **diagram of the different origin point of the ramus septalis**. 1 = *arteria coronaria dextra*, 2 = *ramus septalis*, 3 = *arteria coronaria sinistra*.

Rabbit Heart

- **AIM** : To determine effect of different drugs on Normal and Hypodynamic Rabbit heart
- **Hypodynamic heart** – defined as the heart exhibiting subnormal power or force than the normal one.
- Experimentally, it is developed by the supply of the $1/4^{\text{th}}$ of Calcium chloride (CaCl_2) than the required one which reduces the heart rate.

- For normal heart – McEwens solution
- For hypodynamic heart – 1/4th of CaCl₂ in McEwens solution



- 1 Carotid trunk
- 2. Systemic trunk
- 3. Pulmo cutaneous trunk
- 4. Precaval vein
- 5. Left auricle
- 6 Truncus arteriosus
- 7 Ventricle
- 8 Post caval vein

Frog heart-Ventral view

Frog Heart

- **AIM** :- To demonstrate the Effect of inotropic and chronotropic effects of various drugs on frog heart
- The heart is the most common site for the drug target.
- Positive inotropic & chronotropic drugs like Adr and NA .
- Negative inotropic & chronotropic drugs like ACh .

Tissues/muscle	Identification point	Drug sensitivity	Receptors
Tracheal chain	Above esophagus	NA, A & Ach	β 2 & M2/M3
Stomach fundus	Upper grey part attached to thick red Pylorus	5-HT > Ach > Histamine > Bradykinin	5HT-D
Ileum	Cecum at middle part & large intestine at distal part	Histamine > Ach	H1, Muscarinic
Ascending colon	4-7 cm from ileocecum junction	NA>A Ach,5-HT	β 3, 5HT-2A, 5HT4
Descending colon	5-7cm above rectum	Ach	Muscarinic
Anococcygeus	Thin muscle strip arises from the sacral vertebrae	NA, Ach, 5-HT, isop, No histamine	Adrenergic supply, NANC
Vas deferens	Attached to epididymis	NA & Ach	α 1, Muscarinic
Uterus	Clear two horn above rectum connected to ovary	Oxytocin,5-HT,A,NA Ach	β , α M2/M3

Rabbit Perfused Ear Artery

- Lop-eared rabbit is used
- Central artery is identified , cannulated , dissected & perfused .
- Used for bioassay of catecholamines



Rabbit Aortic Strips

- Descending thoracic aorta is used .
- Aorta is cut in a close spiral .
- 2 to 4 cms of strips are used for experiment .
- Has a very slight spontaneous tone .
- Shows no rhythmic contraction .
- Responds to Adr and NA.

Tissue / Organ	Response	Receptor
Rat isolated vas deferens and anococcygeus Rabbit and guinea pig isolated aorta Rabbit isolated jejunum Guinea pig isolated ileum	Contraction Contraction Inhibition Inhibition	Postsynaptic α_1
Rabbit isolated ear artery and jejunum	Inhibition	Presynaptic α_2
Rabbit isolated perfused heart Rabbit isolated jejunum	Contraction Inhibition	β_1
Guinea pig isolated trachea Rat isolated uterus	Inhibition Inhibition	β_2
Mouse isolated spleen	contraction Relaxation	Both