most likely be needed for establishing the mechanism of PO_2 sensing. Some of the most sensitive systems linked to changes in basal ROS generation by PO_2 are the metabolizing systems for ROS that have regulatory interactions with cellular signaling mechanisms.[1–3] For example, the metabolism of peroxide by the heme peroxidase activity of cyclooxygenase oxidizes the heme of this enzyme into the redox state needed for producing prostaglandins. Glutathione peroxidase generates oxidized GSH while metabolizing peroxide, and oxidized GSH is used as a substrate for the regulation of proteins through *S*-thiolation. The metabolism of peroxide by catalase can be linked to the stimulation of soluble guanylate cyclase and increases in cGMP. The subcellular localization of oxidases whose activity is controlled by PO_2 can influence which mechanism ROS production is linked to. Thus, the actions of exogenously generated ROS may not exactly match endogenously produced species. Oxidases involved in eliciting PO_2-mediated responses may change with physiological or cell culture conditions, which alter oxidase activity or expression. Thus, cellular and molecular approaches for elucidating PO_2 sensors used for regulation physiological regulation *in vivo* need to be designed carefully to consider the impact of changing oxidase activities and the organization of signaling processes linked to PO_2 sensors.

[11] Survival Surgery for Coronary Occlusion and Reoxygenation in a Rodent Model

By WILLIAM A. WALLACE, ARTURO J. CARDOUNEL, SAVITA KHANNA, and CHANDAN K. SEN

Introduction

In order to better understand the pathophysiology and to develop new treatment regimens in myocardial infarction and heart failure, animal models, which simulate human disease, are necessary. Development of animal models based on survival surgery allows the study of tissue remodeling in response to a specific insult. The importance of having a model that is clinically relevant underlies the attractiveness of the widely used coronary artery ligation model in the rat in which the left anterior descending artery (LAD) is temporarily occluded. The progression of heart failure in these rats is similar to the clinical syndrome of heart failure following anterior wall myocardial infarction. LAD occlusion results in acute myocardial ischemia with LV dysfunction, decreased contractility, and elevated

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filling pressures. Reduction in blood pressure, cardiac output, and stroke volume suggest forward failure with LV dilatation and hypertrophy.[1–4] In addition to the correlations of this model to human disease, pharmacological intervention following LAD occlusion in the rat has been shown to be a useful tool in determining drug efficacy.[5] Therefore, temporary LAD occlusion in the rat serves as a relatively simple and low-cost model for studying the pathophysiology of acute myocardial infarction and secondary heart failure, with clear correlation to the clinical setting. This article describes a simplified approach to study the long-term effects of ischemia–reperfusion in the rat heart. The procedure to conduct such survival surgery with a degree of success and efficiency is described.

Procedure

Male Sprague–Dawley rats (Harlan–Sprague–Dawley) weighing 350 g and greater are used. The procedure itself consists of preoperative anesthesia, endotracheal intubation, left thoracotomy, occlusive ligation of the left anterior descending coronary artery, release of LAD ligature, chest tube placement, thoracotomy closure, extubation, and removal of the chest tube.

Preoperative Anesthesia

The rat is placed in an acrylic induction chamber (Harvard Apparatus, NP 60-5246) and is administered halothane or isofluorane until unconscious. It is then transferred to a heated small animal operating table (Harvard Apparatus, AH 50-1239). The operating table is prewarmed to the 1.5 setting. The rat continues to receive anesthesia delivered via a table-top anesthesia machine and vaporizer (Harvard Apparatus, NP 72-3011; NP-72-3038) via spontaneous respiration through a nose cone. Our laboratory uses a medical air cylinder (21% O_2) to deliver the anesthetic. Some laboratories opt to use pure oxygen instead. If pure oxygen is used, it is important to observe that cardiac biology may be altered by exposure to perceived hyperoxia.[5] The vaporizer is initially opened to its highest setting,[6] but is titrated down to 2.5 over approximately 1 min.

At this time, while the animal continues to breathe anesthesia, it is positioned and shaved. The rat is placed in a supine position, securing the hind

[1] D. Elsner and G. A. Riegger, *Curr. Opin. Cardiol.* **10**(3), 253 (1995).
[2] P. Anversal *et al.*, *J. Clin. Invest.* **89**(2), 618 (1992).
[3] J. M. Hagar, R. Matthews, and R. A. Kloner, *J. Am. Soc. Echocardiogr.* **8**(2), 162 (1995).
[4] J. Kajstura *et al.*, *Circ. Res.* **74**(3), 383 (1994).
[5] S. Roy, S. Khanna, A. A. Bickerstaff, S. V. Subramanian *et al.*, *Circ. Res.* **92,** 264 (2003).
[6] S. Goldman and T. E. Raya, *J. Card. Fail.* **1**(2), 169 (1995).

limbs and tail to the table with tape. The animal's left thorax is shaved with a clipper using a #40 blade (Harvard Apparatus, NP 52-5204), taking care to clean away any excess fur. The time necessary for this preparation should provide sufficient anesthesia for attempting endotracheal intubation of the rodent.

Endotracheal Intubation

This step is technically challenging and should be mastered prior to its use in the coronary occlusion and reperfusion protocol. Due to the dimensions of the rodent's anatomy, surgical loupes are recommended to assist in this step (Designs for Vision, Inc, 2.5X). For our purposes, the rat is approached in a prone, suspended position. Using a claw stand (Harvard Apparatus, AH 50-4589), the rat is suspended by its superior incisors from a loop of 2-0 silk suture from the claw stand, allowing the jaw to open (Fig. 1). Using curved forceps in the right hand, grasp the rodent's tongue and retract to your right. In your left hand, a laryngoscope (Harvard Apparatus, AH 59-6581) with a Miller 0 blade (Harvard Apparatus, AH 59-6771) is inserted into the rat's oral cavity to illuminate the pharynx (Fig. 2). The epiglottis may not be visible at this step.

An arrow radial artery catheterization set (Harvard Apparatus, RA-04020) is used for the endotracheal stylus. The 20-gauge angiocath provided in the set is replaced with a 16-gauge polyurethane angiocath (1 3/4 in.). The angiocath is advanced past the needle tip to prevent injury to the animal's oropharynx, and the spring-wire guide is advanced past the angiocath tip to act as a stylus for passage between the vocal cords (Fig. 3).

Replace the forceps in your right hand with the endotracheal intubation set described in the preceding paragraph. Insert the angiocath with the spring-wire guide extended past the tip into the oral cavity to the pharynx. Press gently on the soft palate of the rat to identify the epiglottis. From the time the rat is suspended to this step, only 15 s should have passed, as the rat is no longer receiving anesthesia. The rat should still be breathing spontaneously, and now with the epiglottis visible, the vocal cords should be visualized opening and closing. Pass the spring-wire guide through the vocal cords, and pass the angiocath over the guide, through the vocal cords, and into the trachea.

Remove the needle and spring-wire guide from the angiocath, taking care not to dislodge it from the trachea. Remove the rat from the silk loop and place back into a supine position. Remove the nose cone breathing tube and connect the angiocath to a small animal ventilator (Harvard Apparatus, NP 55-3438). Initial settings for positive pressure ventilation include a stroke volume of 12 ml/kg and a respiratory rate of 70 cycles/min.

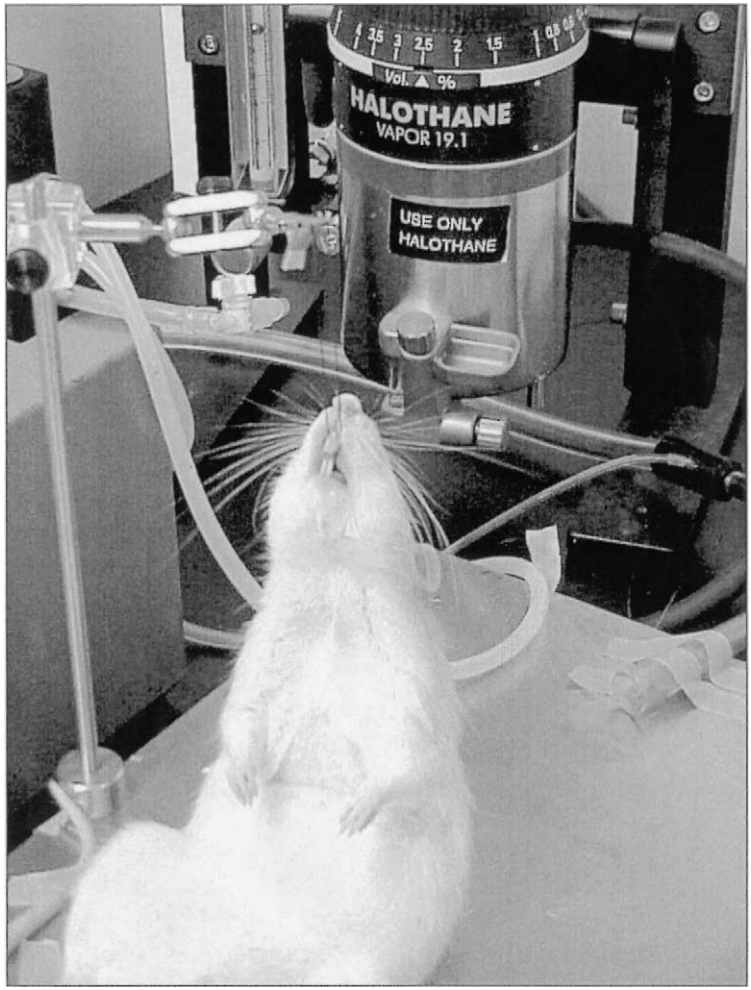


FIG. 1. Positioning of rat for endotracheal intubation.

Connect the anesthesia machine to the ventilator inflow to provide endotracheal intubation. Feel the chest of the animal to ensure adequate filling of the lungs, while simultaneously inspecting the animal's left upper abdominal quadrant to exclude inadvertent esophageal intubation. Inadvertent esophageal intubation would result in gastric dilatation, ineffective ventilation and anesthesia, and great risk for profound hypoxia to the animal. Once proper positioning of the endotracheal tube is verified, secure

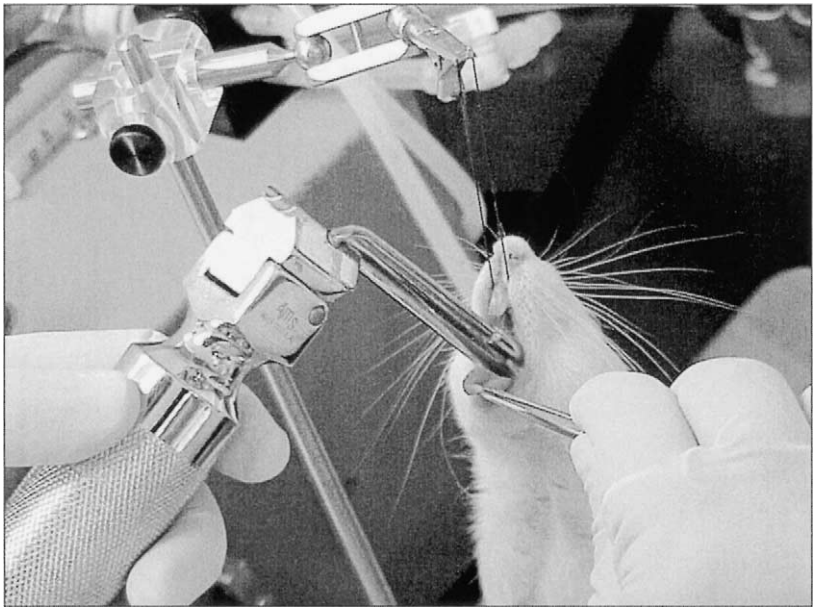
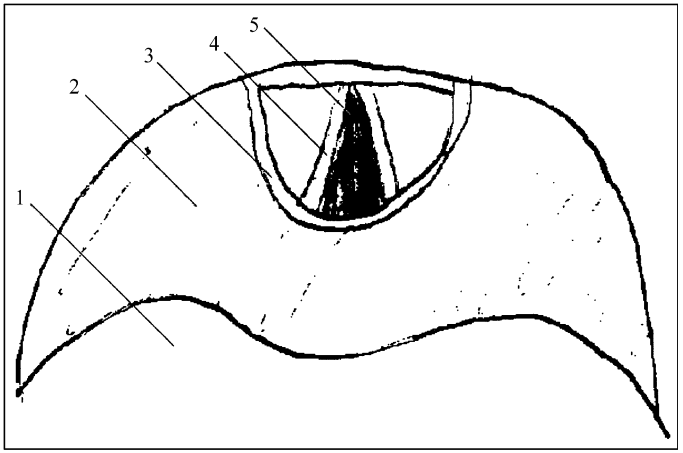


FIG. 2. Visualization of oropharynx for endotracheal intubation.



1. Tongue 2. Esophagus 3. Epiglottis 4. Vocal cord 5. Trachea

FIG. 3. Illustration of rat oropharynx. Adapted from *A Color Atlas of Anatomy of Small Laboratory Animals, Volume Two: Rat, Mouse, Hamster*, 1990 Wolfe Publishing.

the endotracheal tube both to the table and to the animal to prevent accidental removal.

Coronary Occlusion and Reperfusion

Use a fiber-optic goose-necked light source (Harvard Apparatus NP 72-0210, NP 72-0267) to illuminate the surgical field. Clean the rodent's chest with povidine/iodine solution. Identify the inferior point of the sternum of the animal (Fig. 4). One centimeter superior to this point make a left anterior transverse thoracotomy, extending toward the animal's left axilla. Medium-weight scissors work well in cutting the skin. Dissect the underlying fascia to identify the pectoralis major. Divide this muscle transversely. Likewise, identify the serratus anterior and pectoralis minor muscles and divide them using dissecting scissors. Identify blood vessels and cauterize them before division to minimize blood loss (Harvard Apparatus, HB 56-1605). At this point you should identify the fourth and fifth ribs and the fourth intercostal space (Fig. 5).

Carefully make a defect in the intercostal muscle superior to the fifth rib, taking care not to injure the underlying lung and heart. Extend the intercostal incision laterally, avoiding the lung. Extend the incision medially, being careful to avoid the internal thoracic artery, located just lateral

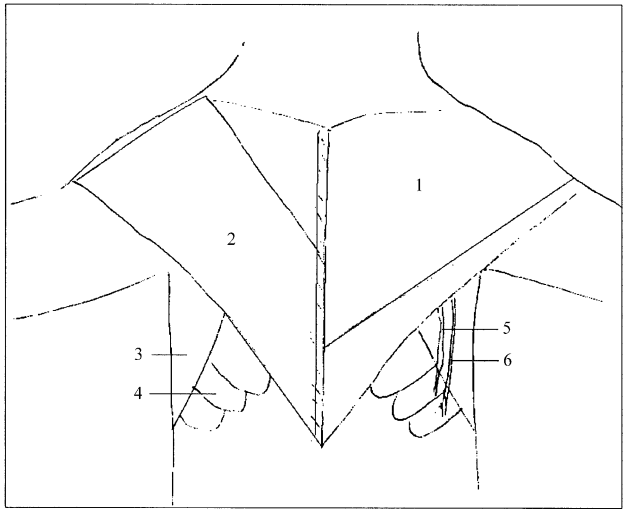


FIG. 4. Illustration of rat chest wall musculature. Adapted from *A Color Atlas of Anatomy of Small Laboratory Animals, Volume Two: Rat, Mouse, Hamster*, 1990 Wolfe Publishing.

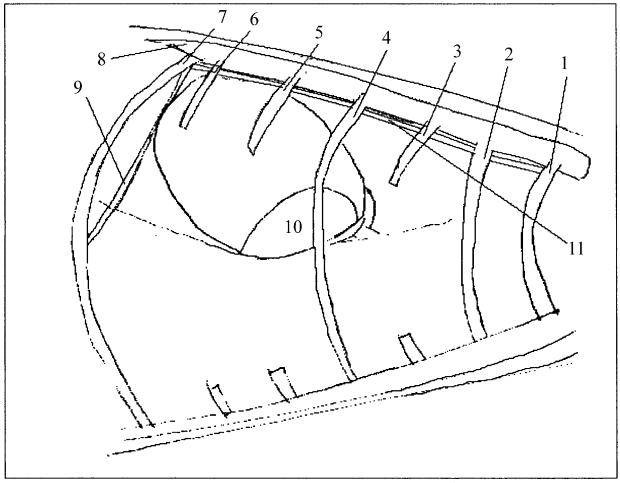


FIG. 5. Illustration of rat thoracic anatomy. Adapted from *A Color Atlas of Anatomy of Small Laboratory Animals, Volume Two: Rat, Mouse, Hamster*, 1990 Wolfe Publishing.

to the sternum interior to the ribs. Insert a rib retractor (Harvard Apparatus, AH 834-149-07) and open to expose the heart. The claw stand used for intubation may be used to hold the retractor to the right, out of the way of the operation.

Elevate the pericardium from the heart, a thin membrane, and open it with sharp scissors. The LAD should be identified extending toward the apex of the heart, originating underneath the left atrial appendage. The left atria can be retracted with a microclip (Harvard Apparatus, NP 61-0186) to aid in exposure of the LAD. Using microneedle holders (Harvard Apparatus, NP 60-3986), place a 6-0 Maxon or equivalent monofilament suture on a T-30 taper needle around the LAD. Care must be taken to place the suture through the myocardium at the proper depth, as too deep can result in hemorrhage and too shallow can result in transaction of the coronary artery. Cut the needle from the suture and place both ends of the suture through a polyethelene tube of approximately 1 cm in length (inner diameter 1.2 mm). Snare the LAD securing the tubing in place using a microclip at the distal end of the tubing to prevent the suture from slipping (Fig. 6).

Time of coronary occlusion begins upon securing of the suture in the polyethylene suture. For our purposes, we allow a period of 30 min to pass,

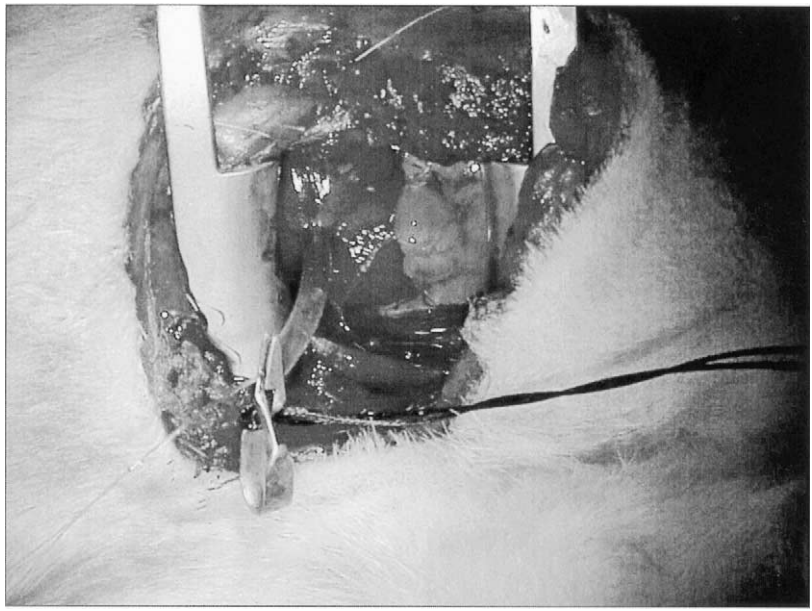


FIG. 6. View of rat heart with occlusion of coronary artery.

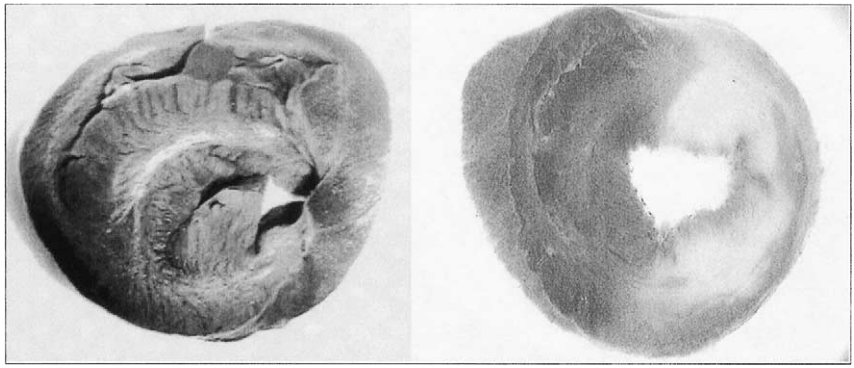


FIG. 7. Cross sections of rat heart without (left) and with (right) ischemia-reperfusion injury.

after which the snare around the LAD is released. This point begins the reperfusion period. The duration of occlusion may be varied depending on specific requirements (Fig. 7).

Thoracotomy Closure

An 18-gauge angiocath (1 3/4 in.) is used as a chest tube with the following modification. Using a 25-gauge needle, the angiocath is pierced three times at the distal tip to allow for additional suction holes. The 18-gauge angiocath is placed through the skin, inferior to the incision, and carefully through the fifth intercostal space, below the incision, into the thoracic cavity. Care must be taken not to injure the heart or lung with the needle. The chest tube is grasped with forceps and brought farther into the thoracic cavity. The angiocath is connected to a 30-ml syringe using a three-way stopcock. Silk suture (2-0) on a T-5 taper needle is used to reapproximate the ribs, placing the suture around the fourth and fifth ribs and tying to secure. Approximately three stitches are necessary for this step.

The muscle layers are then approximated, again using 2-0 silk suture. Reapproximate the deep layers (pectoralis minor, serratus anterior) and then the superficial pectoralis major muscle. Use 9-mm wound clips (NP 34-0554, NP 34-0555) to close the skin. Evacuate the thoracic cavity by aspirating the syringe attached to the chest tube. Use the stopcock to maintain negative pressure in the thoracic cavity when emptying out the syringe. Repeat this procedure as necessary (our laboratory does this twice after closure of the skin and an additional time after the endotracheal tube is removed).

Turn off the anesthesia, but continue ventilating the animal. As the anesthesia wears off, the rodent will begin to breathe spontaneously over the minute ventilation provided by the ventilator. Detach the endotracheal tube from the ventilator, ensuring that the rodent continues to breathe spontaneously. If the animal stops breathing, simply reconnect the endotracheal tube to the ventilator and allow for more recovery time. If the rodent continues to breathe spontaneously, remove the endotracheal tube and evacuate the chest cavity once more, withdrawing the chest tube as you aspirate. Untape the rat's extremities and transfer it back to its cage.

Postoperative Monitoring

The rat will continue to sleep for approximately another 30 min. Check on the animal every 10–15 min until it wakes up fully. Buprenorphine (0.05–2.5 mg/kg sc) injected intramuscularly every 12 h for 24–48 h is usually sufficient as an analgesic. The rat will begin using its left front extremity after approximately 24 h of the surgery. Observe the rat for every hour for approximately 4 h after recovery. Look for signs of pain and/or discomfort, including labored breathing and increased vocalization. The animal may then be safely transferred back to its normal housing facility. Our facility is a climate-controlled environment with 12-h light/dark cycles.

Water and standard rat chow should be provided *ad libitum*. Observe the animals at least twice daily for evidence of labored breathing, decreased food/water intake, or decreased alertness (blunted response to stimuli). Daily monitoring should occur until such time as the surgical clips are removed (7–10 days) or until the heart is harvested. If postoperative complications arise, the animal should be euthanized properly via CO_2 inhalation.

Harvesting of Tissue

After the period of reperfusion has passed, euthanize the rodent and reopen the original incision. Take care not to injure the heart as early adhesions may have already begun forming. Extend the incision across the midline to facilitate removal of the heart. Using scissors, remove the heart from the chest. Using a #10 blade scalpel, remove the injured area of the left ventricle. Typically, there is a small pale area of myocardium where the injury occurred. An area of local intramyocardial hemorrhage where the suture was placed is observed infrequently.

[12] Measurement of *In Vivo* Oxidative Stress Regulated by the Rac1 GTPase

By MICHITAKA OZAKI and KAIKOBAD IRANI

Introduction

The reduction–oxidation (redox) state is emerging as one of the most important determinants of cellular and organ function. The redox status of cells, and ultimately whole tissue, is dependent on a balance between reducing and oxidizing agents within cells. The reducing properties of the cell are affected by the expression of antioxidant enzymes such as glutathione, catalase, thioredoxin, and superoxide dismutase, among others. In contrast, reactive oxygen species (ROS) generated both extracellularly and intracellularly act as oxidizing agents and include superoxide, hydrogen peroxide, and the hydroxyl anion. These ROS by themselves, or secondary species derived from chemical interactions among one another, are primary determinants of the oxidation side of the cellular redox equation. Production of ROS above the antioxidant buffering capacity of the cell results in oxidative stress.

Although the existence of ROS has been known for a long time, their role as signaling intermediaries has only recently come to light. Originally thought of as toxic by-products of aerobic metabolism that indiscriminately

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