

# BLOOD COLLECTIONS

*by* Iwan Sahrial Hamid

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## BLOOD COLLECTIONS

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### IN MOUSE

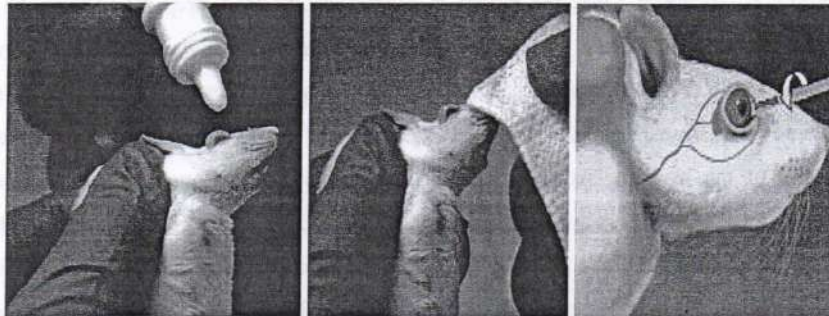
It is important to select the proper method of blood collection that corresponds to the volume required for your research purposes. Some methods are intended for survival and others are not.

#### Retro-orbital Sinus Blood Collection

The retro-orbital sinus is the site located behind the eye at the medial or lateral canthus. This venous sinus is located just underneath the conjunctival membrane. This method is intended for survival blood collection. No more than 10% of the blood volume should be removed at one sampling. The blood volume of a mouse is approximately 8% of the body weight. For example, a 25 gram mouse has a blood volume of approximately 2 ml, so no more than 200  $\mu$ l of blood can be removed at a single bleeding without scientific justification and approval of the Animal Care and Use Committee. Mice should not be bled more frequently than every 3 weeks unless smaller volumes are collected.

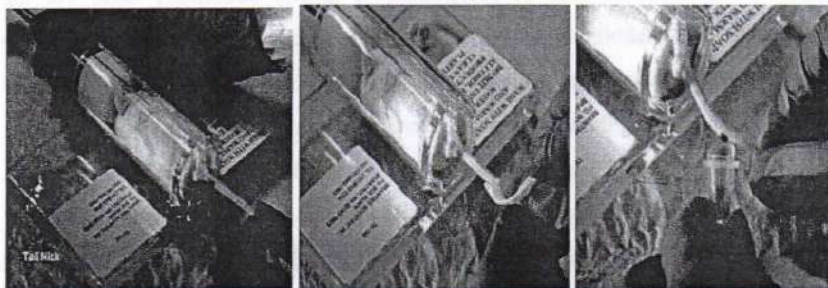
Restrain the mouse by the scruff method. It is imperative that the mouse be properly restrained. If the mouse is allowed to move its head, severe injury to the eye or surrounding tissues could occur. A topical ophthalmic anesthetic must be used prior to performing this procedure. Apply one drop of an anesthetic such as proparacaine or tetracaine hydrochloride to the eye. Be careful not to touch the tip of the applicator to any part of the mouse. This will cause contamination of the anesthetic. Wait 5 - 10 seconds after the anesthetic is applied before attempting this procedure. Gently blot away excess anesthetic with a clean gauze pad, being careful not to scratch the cornea. An alternative to topical anesthesia for this procedure is general anesthesia. With a gentle rotating motion, insert the tube through the sinus membrane. Continue rotating the tube at the back of the orbit until blood flows. Collect the appropriate volume of blood. Upon completion, ensure good hemostasis with a clean gauze pad before returning the animal

to its cage. Be careful not to scratch the cornea with the gauze pad. To become proficient at this technique, additional training outside the scope of this text is required.



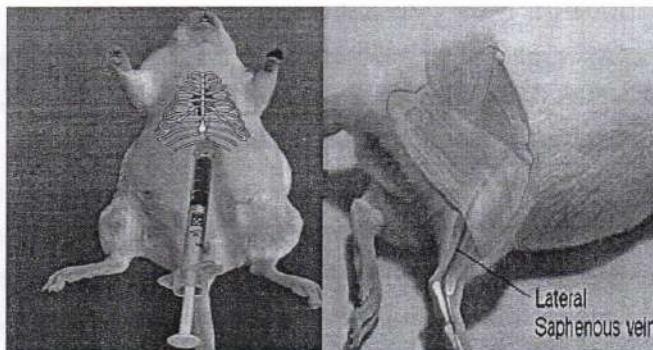
#### **Blood Collection Via the Lateral Tail Veins**

Tail nicking is a survival procedure that can be used to collect up to 200  $\mu$ l of blood from the lateral tail veins. This method must be used with caution, as when improperly performed, permanent tail injury or amputation may occur. Warm the animal under a heat source, being careful NOT to overheat. The temperature at the level of the animal should not exceed 85 - 90° Fahrenheit. Place the mouse in a restrainer. Prep the tail with 70% ethanol. Stabilize the tail with the thumb and forefinger of the hand that will not be used to nick the tail. Using a #11 scalpel blade, gently nick the lateral tail vein in the general area around the midline of the tail. Start at least half way down the tail so that if there is a problem, you can nick the tail above the initial site and still obtain your blood sample. Allow the blood to flow into an appropriate receptacle. Do not squeeze the tail or attempt to milk blood from the tail. This may cause tissue damage and contamination of the blood sample with tissue fluids.



### Intracardiac Puncture

Intracardiac puncture must be performed under deep anesthesia and is considered a nonsurvival procedure. Once the mouse is deeply anesthetized, prep the ventral chest area with 70% ethanol. When an appropriate volume has been collected, ensure good hemostasis with a dry, sterile gauze pad, surgical glue or silver nitrate. Insert the needle at the base of the sternum, bevel up, into the thoracic cavity at a 15 - 20° angle directed just to the left of the midline. Aspirate slowly. If blood starts to flow into the syringe, continue to aspirate with steady, even pressure. If no blood is seen, reposition the needle and attempt aspiration. Once the required blood volume is collected, the mouse is euthanized while still deeply anesthetized. Up to one milliliter or more of blood may be collected from an adult mouse using this method. Alternatives to the methods described here include collecting blood from the saphenous vein. A description of this method can be found in the reference section.



### IN RABBITS

Intravenous administration of compounds is mostly commonly done via the marginal ear vein. Most rabbits must be sedated for this procedure. The hair over the vein should be carefully clipped. The vein can be dilated by applying methylsalicylate or warm water or by occluding the vessels at the base of the ear. Carefully insert the needle - with the bevel facing up and parallel to the vessel - into the vein and slowly inject the compound. If the vessel blanches or clears of blood, the material is going intravascularly. If a bleb forms at the injection site, the material is not going into the vessel. Following injection and removal of the needle (23-gauge, butterfly catheter), gentle pressure should

be applied with dry gauze for 1 to 2 minutes to prevent hematoma formation. If a vasodilator is used, clean the area with water to avoid skin irritation. Other less common sites include the lateral saphenous and external jugular veins.

The auricular artery is often used to collect blood samples in rabbits. It can easily be seen on the dorsal surface of the ear in the center between the two marginal ear veins. As a general rule, it is safe to withdraw about 20% of an animal's blood volume at weekly intervals (blood volume in liters = 6% of body weight in kilograms). Animals in which larger quantities of blood are removed should be monitored frequently to ensure that the animal is replacing its blood components. As for IV injections, rabbits should be sedated beforehand. Hair on the ear should be gently clipped. The vessel may be dilated by applying warm water or methylsalicylate solution to the skin overlying the vessel. Using a 23-g butterfly catheter or 21-gauge needle with the hub removed, one can easily collect up to 30cc of blood at one time. The needle should be inserted with the bevel facing up and oriented parallel with the artery only a few millimeters. After removing the needle, apply firm pressure to the artery to avoid hematoma formation.; then, if methylsalicylate was used, wash the ear completely to avoid damage to the skin.

**Cardiac Puncture** - To collect large samples of blood, cardiac puncture can be performed, but only on a fully anesthetized animal and as a terminal procedure. This technique is commonly used for exsanguination of rabbits that are used for polyclonal antibody production. The anesthetized rabbit is placed on its back and a 20- or 22-g needle with syringe or similar sized vacutainer needle is placed through the abdominal wall, lateral to the xiphoid process. The needle is angled 10-30° above the horizontal plane and slowly advanced. Once the heart is contacted, you will feel the syringe move with the beating of the heart. Between 90 -120cc of blood can usually be obtained with this technique.

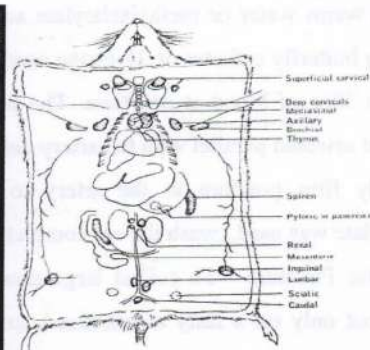
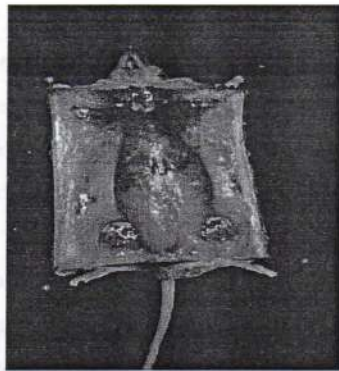
## Necropsy of the Mouse

### 1. Subcutaneous

The presence of a glossy, gelatinous and clear liquid filled subcutaneous space confirms the diagnosis of oedema, Small, more or less numerous, haemorrhagic petechia can moreover be observed more frequently in mice affected by an infectious disease.

### 2. Superficial and deep lymph nodes

In normal conditions, the lymph nodes of the mouse are easily detectable. They are numerous, of variable size in different strains of animals, greyish, and shaped as a small pea or bean.

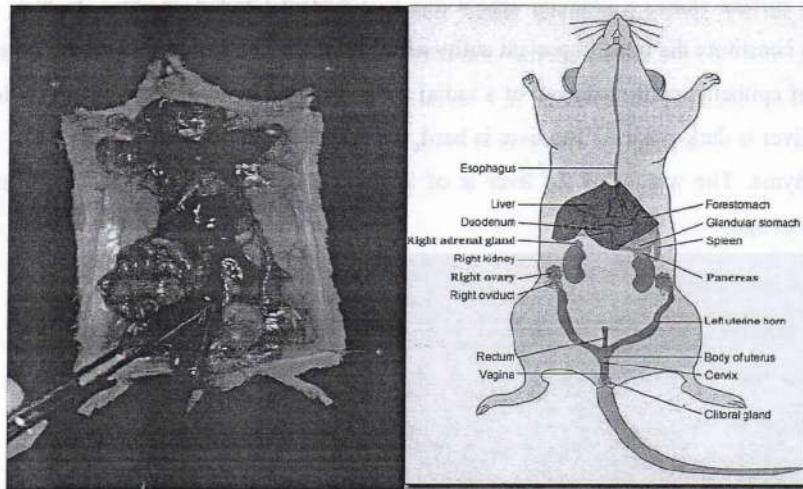


Opening of the mouse skin: external examination of subcutaneous tissues, muscles, lymph nodes and mammary glands

### 3. Spleen

The spleen is an organ of predominantly lympho-erythropoietic function. The parenchyma of this organ contains: a) a tissue with erythropoietic function called red pulp, constituted by vessels and cords of various types of red cells (haemocytoblasts, erythrocytes, leukocytes, megakaryocytes); b) a lymphoid tissue called white pulp.

The spleen is situated in the left superior abdominal quadrant; it has a lengthened, oval, slightly curved shape. In a young-adult mouse the spleen measures approximately 15 millimetres in length, 3 millimetres in width, 2 millimetres in thickness. Its average weight is approximately of 100 mg.



Spleen extraction

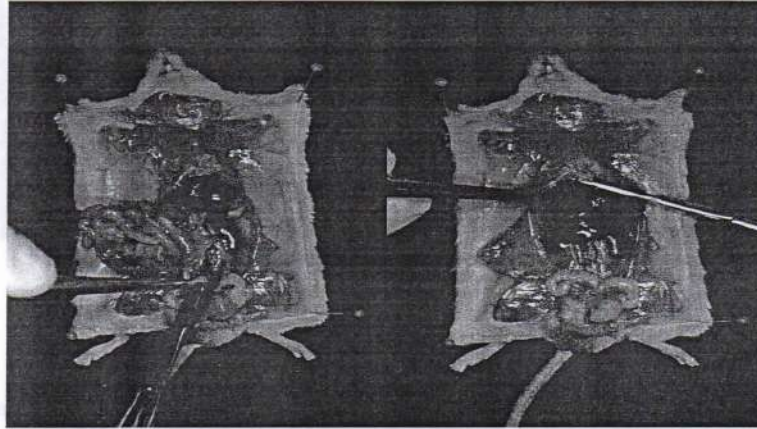
### 3. Pancreas

The glandular pancreatic tissue in the mouse is completely enclosed in the mesenteric adipose tissue and does not appear as true compact organ as in the human being. Frequently, in the old animals, the pancreas is atrophic, because it is completely lacking in acini. The atrophy of the pancreas can also be the consequence of a chronic pancreatitis or nodular polyarteritis. In some cases, the pancreatic ducts can be found enlarged as cysts due to stenosis of the pancreatic duct, and its rupture may cause a disease such as fat necrosis (steatonecrosis).

### 4. Liver and biliary ducts

The liver is a large glandular organ which occupies a large portion of the abdominal cavity of the mouse. With its superior convex surface, the liver adheres to the diaphragm, while its inferior concave surface is in contact with the stomach and the duodenum. The liver has four large lobes which join themselves in the dorsal region around the hilum. It can be distinguished a median lobe, two lateral (one right and one left), and one caudal lobe, subdivided into dorsal and ventral half. A thin transparent capsule, called the Glisson capsule, covers the organ. At the macroscopic examination,

the cut surface shows a granular aspect due to a typical lobular structure. In fact, the lobules constitute the most important entity of the liver and they are composed by several cords of epithelial cells, arranged in a radial pattern around a vessel. The normal colour of the liver is dark-reddish. The liver is hard, but friable when one exerts pressure on the parenchyma. The weight of the liver is of approximately 1.34 g in a mouse of three months of age.



Stomach and gut extraction

Liver extraction

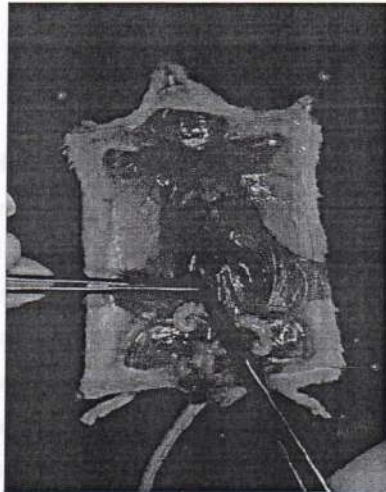
The cholecyst, also called gall bladder, is visible on the inferior surface of the organ where it appears like a small bag of a few millimetres in diameter. The bile, produced by the liver, reaches the gall bladder via the hepatic duct, where it is concentrated; the bile reaches the intestine through the cystic and the common bile ducts.

## 5. Urinary apparatus

The urinary apparatus consists of kidneys, ureters, bladder and urethra. The variation in dimensions or position of these organs might be easily appreciated by comparing the two kidneys. In fact, the right is usually larger and heavier than the left one, and it is also positioned more cranially. The weight of the right kidney is approximately 210 milligrams, and that of the left is 200 milligrams. The kidneys show a hard compact consistency, and a reddish colour that sometimes turns to yellow. The ureters are two thin small ducts that connect the kidneys to the bladder. Their main



function is to allow the urine, produced by kidneys, to be collected in the bladder. The ureters end up separately in the posterior part of the bladder, close to the neck. The bladder appears as a small oval bag, covered by a thin greyish wall, and lies in the anteromedial area of the abdominal cavity. In its inferior part, this bag shrinks and continues in the neck and then in the urethra.



Kidney and ureter extraction



Uterus Removal

## 6. Female genital apparatus

The genital apparatus of the female mouse includes the uterus, the ovaries, the oviducts and the vagina. The uterus is formed by a tubular median part (body of the uterus) and two lateral formations (lateral horns). The caudal part of the uterus body is called the neck. The uterus is found in the inferior part of the abdominal cavity and adheres to the posterior wall of the abdomen. In particular, the body of the uterus is placed anteriorly to the urinary bladder and is covered by a serosa adherent to the posterior wall of the abdomen. In normal conditions the colour of the organ is greyish. The ovaries lie close to the inferior pole of the kidneys and are tied to the posterior wall of the abdomen by means of the mesovarium. They are small bilateral spherical organs with smooth surfaces. During the period of the sexual maturity, the ovaries assume a nodular aspect due to the presence of follicles and corpora lutea.

Beyond their role as endocrine glands, the ovaries have the fundamental function of production of the oocytes that, once expelled, pass into the uterus through the oviducts, and there are fertilized. The oviducts are two tight and small tubes, wrapped around themselves as a ball; they connect the ovaries to the horns of the uterus. Finally, the vagina is a short channel that begins from the neck of the uterus and ends externally in front of the anal opening. In the abdominal cavity, the vagina lies in front of the rectum and behind the urethra.

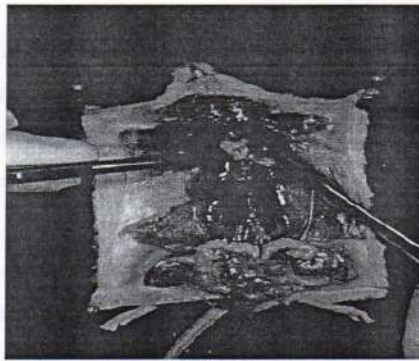
## <sup>6</sup> 7. Lungs and heart

**The lungs** are two large organs of the thoracic cavity and are composed of lobes. The right lung of the mouse, like that of all other small rodents, is larger than the left one. This is due to the fact that the heart occupies, with its diameter, the greater part of the left thoracic cavity. While the right lung is composed of four lobes (the superior, the mean, the post-caval, and the middle), the left lung shows a single lobe. In normal conditions, the colour of the lung depends on the amount of air and blood present, usually it is pale rose-coloured. The lungs are elastic and have a spongy consistency. A thin and transparent serosa membrane, called visceral pleura, covers the lungs. At the level of the median wall, in proximity of the hilum of the lung, the pleura is reflected and covers the inner wall of the thoracic cavity and then becomes the parietal pleura. A thin space is present between these two membranes (pleural cavity).

**The heart** has a pyramidal triangular shape, with its greater axis oriented obliquely to the left. It is divided, by the septum, in one left and one right half with no communication between them. An atrium and a ventricle form each half of the heart. The blood from the atrium goes into the ipsilateral ventricle and then into the circulation, either pulmonary on the right hand side or systemic on the left. The atrioventricular canals are supplied with a valve that allows the blood to flow from the atrium to the ventricle, but not vice versa. The <sup>15</sup>tricuspid valve lies between the right atrium and the ventricle; the bicuspid valve is found between the left atrium and the ventricle. T

Through the vena cava, the non-oxygenated blood arrives in the right atrium and then passes in the ipsilateral ventricle. Through the pulmonary artery, the blood reaches the lungs where it is oxygenated, and it returns to the left atrium, through the pulmonary veins; then it goes into the ipsilateral ventricle and finally it is distributed to all regions of the body through the aorta.

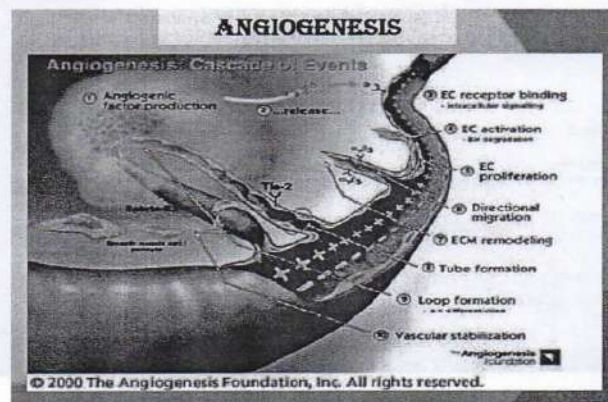
In the mouse, the length of the apical-basal diameter of the heart is of approximately 9 millimetres, while its transverse diameter, at the level of the ventricles, is approximately 5-6 millimetres. A serosal membrane called the pericardium covers the heart; the heart is of reddish colour and weighs around 130mg.



Lungs and heart observatio

## ANGIOGENESIS MODEL

### Angiogenesis Mechanism



1. Diseased or injured tissues produce and release angiogenic growth factors.
2. The angiogenic growth factors bind to specific receptors located on the endothelial cells (EC) of nearby preexisting blood vessels.
3. Once growth factors bind to their receptors, the endothelial cells become activated. Signals are sent from the cell's surface to the nucleus. The endothelial cell's machinery begins to produce new molecules including enzymes.
4. Enzymes dissolve tiny holes in the basement membrane surrounding all existing blood vessels.
5. The endothelial cells begin to divide, and they migrate out through the dissolved holes of the existing vessel.
6. Specialized molecules called adhesion molecules, or integrins (avb3, avb5) serve as grappling hooks to help pull the new blood vessel sprout forward.
7. Additional enzymes (matrix metalloproteinases, or MMP) are produced to dissolve the tissue in front of the sprouting vessel tip in order to accommodate it. As the vessel extends, the tissue is remolded around the vessel.
8. Sprouting endothelial cells roll up to form a blood vessel tube.
9. Individual blood vessel tubes connect to form blood vessel loops that can circulate blood.
10. Finally, newly formed blood vessel tubes are stabilized by specialized muscle cells (smooth muscle cells, pericytes) that provide structural support.

**Chemical stimulation of angiogenesis is performed by various angiogenic proteins, including several growth**

Stimulator	Mechanism
VEGF	Promotes proliferation & differentiation of endothelial cells, smooth muscle cells, and fibroblasts
VEGF	Affects permeability
VEGFR and NRP-1	Integrate survival signals
Ang1 and Tie2	Stabilize vessels
PDGF (BB-homodimer) and PDGFR	recruit smooth muscle cells
TGF- $\beta$ , endoglin and TGF- $\beta$ receptors	Extracellular matrix production
MCP-1	
Integrins $\alpha_v\beta_3$ , $\alpha_v\beta_5$ ( $\alpha_v\beta_3$ ) and $\alpha_5\beta_1$	Bind matrix macromolecules and proteases
VE-cadherin and CD31	endothelial junctional molecules
sphR11	Determine formation of arteries or veins
plasminogen activators	remodels extracellular matrix, releases and activates growth factors
plasminogen activator inhibitor-1	stabilizes nearby vessels
NOS and COX-2	
AC133	regulates angiogenic differentiation
131/113	Regulates endothelial

The fibroblast growth factor (FGF) family with its prototype members FGF-1 (acidic FGF) and FGF-2 (basic FGF) consists to date of at least 22 known members. Most are 16-18 kDa single chain peptides. aFGF (FGF-1) and bFGF (FGF-2) are important players in wound healing. They stimulate the proliferation of fibroblasts and endothelial cells that give rise to angiogenesis and developing granulation tissue, both increase blood supply and fill up a wound space/cavity early in the wound healing process. FGF-2 is a more potent angiogenic factor than VEGF or PDGF (platelet-derived growth factor), however, less potent than FGF-1.

#### **The principal methods now in use:**

Most studies of angiogenesis inducers and inhibitors rely on various models, both in vitro and in vivo, as indicators of efficacy. In this report we describe the principal methods now in use: the in vivo Matrigel plug and corneal neovascularization assays, the in vivo/in vitro chick chorioallantoic membrane (CAM) assay, and the in vitro cellular (proliferation, migration, tube formation) and organotypic (aortic ring) assays. We include description of two new methods, the chick aortic arch and the Matrigel sponge assays.

#### **The Chick Embryo Chorioallantoic Membrane (CAM)**

The CAM is an extraembryonic membrane formed on day 4 of incubation by fusion of the chorion and the allantois. Since it mediates gas exchanges with the extraembryonic environment until hatching, it has a very thick capillary network that forms a continuous surface in direct contact with the shell. Rapid capillary proliferation continues until day 11; the mitotic index then declines just as rapidly, and the vascular system attains its final arrangement on day 18, just before hatching.

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PAGE 1

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PAGE 2

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PAGE 3

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PAGE 4

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PAGE 5

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PAGE 6

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PAGE 7

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PAGE 8

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PAGE 9

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PAGE 10

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PAGE 11

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PAGE 12

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PAGE 13

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