全国高等医药院校药学类实验教材

生物药剂学实验

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内 容 提 要

本书为全国高等医药院校药学类实验教材之一。全书分为九个实验，旨在利用简单的体内外方法评价药物体内四个过程吸收、分布、代谢和排泄，并阐明药物动力参数的计算方法。为适应教育国际化的要求，增加了英文对照内容，以便学生在阅读英文文献、撰写英文论文时参考。

本书可作为药学类实验双语教材及相关专业教材使用，也可供药学类相关人员参考学习。

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前　言

生物药剂学实验对药学教育具有重要的意义。生物药剂学是研究药物及其制剂在生物体内的变化过程的一门课程。生物药剂学实验主要针对药物及其制剂在体内的吸收、分布、代谢和排泄过程以及药物的生物利用度进行试验设计，以便合理设计制剂，为科学制定给药方案打下基础。

本实验教材是根据高等医药院校实验教学的新的发展需要，适应生物药剂学的教学要求与学科发展，内容包括药物的吸收、蛋白结合、代谢、消除等动态过程原理和实验方法以及常用的药动学程序应用等，重点放在基本理论、基础知识和基本技能的理解与掌握。

本教材是药学类实验双语教材，可供药学类相关专业使用，亦可作为研究生和药剂工作者的参考书。

著者在编辑过程中难免有疏漏之处，敬请指正。

编者
2014 年 1 月

contents

目录

实验一　单隔室模型模拟实验

【实验目的】

1. 掌握单隔室模拟的试验方法。
2. 掌握用“血药浓度”、“尿排泄数据”计算药物动力学参数的方法。

【实验指导】

1. 血药浓度　若药物在体内的分布符合单室模型特征，且按表观一级动力学从体内消除，则快速静脉注射时，药物从体内消失的速度为：

$$\frac{dX}{dt} = -kX \tag{1}$$

式中 X 为静注后 t 时刻体内药量，k 为该药的表观一级消除速度常数。将（1）式积分得：

$$X = X_0 e^{-kt} \qquad (X_0：静注剂量) \tag{2}$$

用血药浓度表示为：

$$C = C_0 e^{-kt} \tag{3}$$

（3）式两边取常用对数，表达式为：

$$\lg C = \lg C_0 - \frac{kt}{2.303} \tag{4}$$

式中 C_0 为静注后初始的血药浓度。以 $\lg C$ 对 t 作图应为一直线。消除速度常数 k 可由该直线的斜率 $-\frac{k}{2.303}$ 而求出。C_0 可以从这条直线外推得到，用截距 C_0 可算出表观分布容积：

$$V = \frac{X_0}{C_0} \tag{5}$$

2. 尿排泄数据　药物的消除速度常数有时也可由尿排泄数据来求算。为此，要求至少有部分药物以原型排泄，考虑到药物从体内消除的途径，有一部分采取肾排泄，另一部分以生物转化或胆汁排泄等非肾途径消除。

设 X_u 为 t 时间尿中排泄药物累积量 k_e、k_{nr} 分别为肾排泄和非肾途径消除的表观一级速度常数。于是消除速度常数

$$k = k_e + k_{nr} \tag{6}$$

则原型药物的排泄速度

$$\frac{dX_u}{dt} = k_e X \tag{7}$$

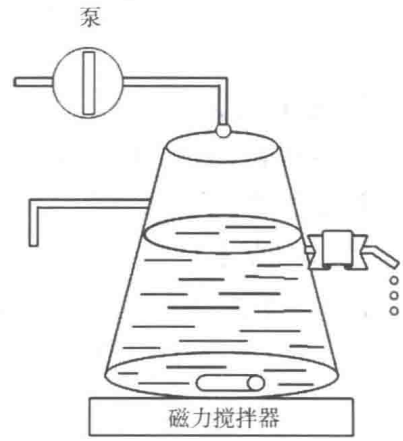
式中 X 为 t 时间的体内药量。将（2）式中 X 值代入（7）式后得

$$\frac{dX_u}{dt} = k_e X_0 e^{-kt} \tag{8}$$

于是

$$\lg \frac{dX_u}{dt} = \lg k_e X_0 - \frac{kt}{2.303} \tag{9}$$

由于实验求出的尿药排泄速度显然不是瞬时速度（dX_u/dt），而是一段有限时间内的平均速度 $\Delta X_u/\Delta t$。这样用 $\Delta X_u/\Delta t$ 代替（9）式中的 dX_u/dt，并以对平均速度的数对时间作图，得一条直线，其斜率为 $-k/2.303$，与血药浓度法所求的斜率相同。故药物的消除速度常数既可从血药浓度也可从尿排泄数据求出。这里要强调一点，平均尿排泄速度对数应该对集尿间隔内的中点时间作图。



【实验内容与操作】

单室模拟装置为带有两支管的三角烧瓶，烧瓶相当于体循环系统。当把药物（用酚红液代替）注入烧瓶后，用蠕动泵将水以一定的流速注入烧瓶中，药物不断地从两支管中清除，两支管清除的药量可看做肾脏清除和非肾脏清除的药量。

1. 操作

（1）将约250ml的蒸馏水倒入三角瓶中，开动磁力搅拌器。用蠕动泵以每分钟约6－8ml的流速将蒸馏水注入烧瓶中，搅拌数分钟，使进入烧瓶中的水量同由两支试管中排出的量相等。用橡皮管与夹子控制其中一个支管的流速3～4ml/min，液体连续滴出，另一个支管间歇流出液体。

（2）用移液管将烧瓶中的水取出10ml，然后用移液管将0.1%酚红供试液10ml加入烧瓶中并计时，此时间记为 t_0。以后每隔10分钟自烧瓶中吸取0.5ml供试液作为“血药样品”，同时定量收集不同时间内较高支管间歇流出的试液作为“尿药样品”供测定用。

2. 定量方法 取0.5ml血药样品（或尿药样品），加入5ml 0.2mol/L的NaOH液，在555nm测定酚红的吸收度并求出浓度。参比溶液：0.2 mol/L NaOH。

3. 标准曲线的制备 精密称取酚红10mg，置100ml容量瓶内，加1% Na_2CO_3液至刻度，配成100μg/ml的标准溶液，分别吸取0.5ml，1ml，1.5ml，2ml，2.5ml，3ml的标准溶液，加水至10ml，按酚红的定量方法测定吸收度并绘制标准曲线。

【实验结果与讨论】

1. 将血药浓度数据和尿排泄数据列于表1、表2：

表1 血药浓度数据

取样时间（分）	10	20	30	40	50	60	70	80
吸收度								
浓度（μg/ml）								

表 2　尿排泄数据

取样时间（min）	10	20	30	40	50	60	70	80
V（ml）								
吸收度								
ΔX_u（μg）								
Δt（min）								
$t_{中}$（min）								

2. 分别用表 1、表 2 两组实验数据作图并计算药物动力学参数。

【思考题】

做好本次实验的关键是什么？在操作中应注意哪些问题？

Experiment 1: One – compartment simulation experiment

Purpose

1. To learn the method for the simulation of one – compartment model.

2. To learn the methods for calculating pharmacokinetic parameters with the plasma drug concentration and urinary excretion data.

Introduction

1. Plasma drug concentration

Given that the distribution process of a drug is consistent with the one – compartment model and the drug is eliminated with apparent first – order kinetics, the elimination rate constant after intravenous administration can be expressed as follows:

$$\frac{dX}{dt} = -kX \tag{1}$$

where X is the existing drug in the body at t hour after injection, k is the apparent first – order elimination rate constant.

Integration of equation (1) gives the following expression:

$$X = X_0 e^{-kt} \tag{2}$$

where X_0 is the intravenously administrated dose.

After both sides are divided by the apparent volume of distribution, equation (2) is expressed in the concentration form.

$$C = C_0 e^{-kt} \tag{3}$$

By transforming equation (3) into its corresponding logarithm form, equation (4) is obtained:

$$\lg C = \lg C_0 - \frac{kt}{2.303} \tag{4}$$

where C_0 is the plasma drug concentration at $t=0$ after administration. As shown by equation (4), there is a linear relationship between lg$C$ and time, and the elimination rate constant k and C_0 can be calculated from the slope ($\frac{kt}{2.303}$) and the intercept respectively. In addition, the apparent volume of distribution can be calculated by equation (5).

$$V=\frac{X_0}{C_0} \tag{5}$$

2. Urinary excretion data

The apparent first-order elimination rate constant can also be determined from the urinary excretion data, but it requires that at least a portion of the intact drug is excreted in the urine. Generally, drugs may be eliminated via both renal route and non-renal routes, such as biotransformation and bile elimination.

If X_u represents the accumulative excretion amount of the intact drug in urine; k_e, k_{nr} represent the apparent first-order elimination rate constant by renal route and non-renal route, respectively, the elimination rate constant k can be calculated by:

$$k = k_e + k_{nr} \tag{6}$$

And the excretion rate of the intact drug in urine can be described as:

$$\frac{dX_u}{dt}=k_eX \tag{7}$$

where X is the amount of drug in the body at t hour.

By substituting equation (2) into equation (7), the following equation can be obtained:

$$\frac{dX_u}{dt}=k_eX_0e^{-kt} \tag{8}$$

Taking logarithmic of both side of the equation,

$$\lg\frac{dX_u}{dt}=\lg k_eX_0-\frac{kt}{2.303} \tag{9}$$

The experimentally determined excretion rate of the intact drug in urine isn't the transient rate (dX_u/dt), but is the mean rate ($\triangle X_u/\triangle t$) during a short time interval. By substituting $\triangle X_u/\triangle t$ for dX_u/dt in equation (9), the plot of lg ($\triangle X_u/\triangle t$) versus t is a straight line, with a slope equal to $-k/2.303$, which is the slope of the linear plot of the logarithm blood drug concentration versus time. Therefore, the apparent first-order elimination rate constant can be determined not only from the plasma drug concentration but also the urinary excretion data. It is important to note that when urinary excretion data used, the logarithm of the mean excretion rate of intact drug in urine should be plotted against the midpoint of each collecting time interval.

Methods

The simulation apparatus of the one-compartment model is consisted of a conical flask with two outlets (upper and lower outlets), a peristaltic pump, and a magnetic stirring plate. In this experimental set-up, the flask can be considered as the central compartment (systemic circulation, Figure 1).

A drug (use phenolsulfonphthalein in this experiment) is added to the flask, with the influx of water via the peristaltic pump, the drug solution is flowing out from the two outlets gradually. The amount of the drug exiting from the two outlets can be regarded as the amount eliminated via the renal route (upper outlet) and non-renal route (lower outlet), respectively.

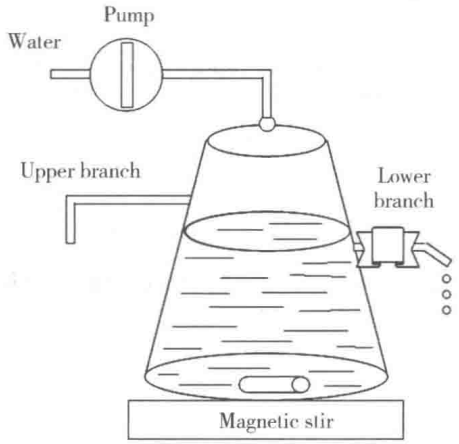


Figure 1. The simulation device of the one-compartment model

1. Procedures

(1) Add 250 ml of water into a conical flask and the magnetic stirring apparatus is turned on with the magneitic stirring bar in the flask. Water is pumped into the flask by the peristaltic pump at a rate of 6 ~ 8 ml/min for several minutes to make sure that the volume of influx water is equal to the volume of outflux water. Using a rubber band or a clamp to make the water flowing out of the upper outlet intermittently while the water flowing out of the lower outlet is continuous a rate of 3 ~ 4 ml/min. (From the diagram, the lower outlet is clamped.)

(2) 10 ml of solution is withdrawn from the flask with a pipette, 10 ml of 0.1% phenolsulfonphthalein solution is added to the flask, and the time is recorded. 0.5 ml of the solution (representing the blood) is taken from the flask for assay every ten minutes, and the solution flowed out from the upper outlet is collected at each interval (representing the urine sample) for quantitation.

2. Quantitative method

To 0.5ml of sample collected, 5 ml of 0.2 M sodium hydroxide solution is added, the absorbance of phenolsulfonphthalein is determined at 555 nm and calculate the concentration using the calibration curve. A 0.2 M sodium hydroxide solution is used as the blank solution for UV measurement.

3. Establishment of calibration curve

A 10 mg of phenolsulfonphthalein standard is accurately weighed, place into a 100 ml of volumetric flask and diluted with 1% sodium carbonate (Na_2CO_3) solution to 100 ml to give a stock solution with a concentration of 100 μg/ml. Different volumes of the standard stock solutions (0.5, 1.0, 0.5, 1.5, 2.0, 3.0 ml) are placed into 10 ml of volumetric flasks and add

distilled water to volume. Measure the absorbance of these standard solutions using the procedures described above and constructs the calibration curve.

Results and Discussion

1. Please record the concentration of phenolsulfonphthalein in Table 1 and Table 2 representing the concentration of a drug in plasma and in urine.

Table 1. Concentration representing a drug in plasma

Sampling time	10'	20'	30'	40'	50'	60'	70'
Absorbance							
Concentration (μg/ml)							

Table 2. Concentration representing a drug in urine

Sampling time	10'	20'	30'	40'	50'	60'	70'
V (ml)							
Absorbance							
$\triangle X_u$ (μg)							
$\triangle t$ (min)							
t_{middle} (min)							

2. Construct PK plots and calculate the pharmacokinetic parameters using the data in Table 1 and 2.

Questions

What are the key factors to be considered in conducting this experiment?

实验二　大鼠在体小肠吸收实验

【实验目的】

1. 掌握大鼠在体小肠吸收的实验方法。
2. 掌握计算药物的吸收速度常数（k_a），以及每小时吸收率的计算方法。

【实验指导】

大多数药物以被动扩散方式从生物膜的高浓度侧向低浓度侧转运。被动扩散可用Fick第一定律来描述。该定律指出，扩散速度（dC/dt）正比于膜两侧的浓度差（ΔC），因此有：

$$-\frac{dC}{dt} = k_a \Delta C = k_a \ (C - C_b) \tag{1}$$

式中 C 是消化道中药物浓度，C_b 是血液中药物浓度，k_a 是吸收速度常数，其值大小取决于药物的扩散常数，吸收膜的厚度与面积及药物对膜的穿透性。胃肠道吸收的生物学过程包括这样一个系统，即药物从胃肠道屏障的一侧（吸收部位）向另一侧

（血液）扩散。因为进入血液的药物很快分布到全身，故与吸收部位比较，血中药物浓度维持在很低的水平。口服给药时，对于胃肠道来说，血液（室）的作用如同一个“水槽”（sink）。整个吸收相保持很大的浓度梯度，$C \gg C_b$，则 $\Delta C \approx C$，于是（1）式可以简化为

$$-\frac{dC}{dt} = k_a C \tag{2}$$

此为一级速度方程式的标准形式。胃肠道按一级动力学从溶液中吸收大多数药物。用消化液中药物量的变化（dX_a/dt）表示扩散速度，则：

$$-\frac{dX_a}{dt} = k_a X \tag{3}$$

将（3）式积分，并在方程两侧同取对数。

$$\ln X_a = \ln X_a\ (0)\ - k_a t \tag{4}$$

式中 X_a 为 t 时间消化液中药物量，X_a（0）为零时刻消化液中药物量，k_a 为药物吸收速度常数。以 $\ln X_a$ 对 t 作图得一条直线，其斜率为药物在小肠中的吸收速度常数（k_a）。

【实验内容与操作】

1. 仪器　蠕动泵；分光光度计；红外灯；手术剪；止血钳；乳胶管；烧杯；固定板；电热恒温水浴锅。

2. 试剂

（1）0.1% $NaNO_2$液。

（2）0.5%氨基磺酸铵（$NH_2SO_3NH_4$）溶液。

（3）0.1%二盐酸萘乙二胺溶液（偶合试剂）；（以上置冰箱保存）。

（4）1 mol/L HCl。

（5）0.2 mol/L NaOH。

（6）生理盐水。

（7）Krobs－Ringer 试液（每 1000ml 内含 NaCl 7.8g，KCl 0.35g，$CaCl_2$ 0.37g，$NaHCO_3$ 1.37g，NaH_2PO_4 0.32g，$MgCl_2$ 0.02g，葡萄糖 1.4g）。

（8）乌拉坦溶液（20% g/g，大鼠每 100g 腹腔注射 0.4ml 麻醉）。

（9）磺胺嘧啶（Sulfadiazine，SD）。

3. 操作

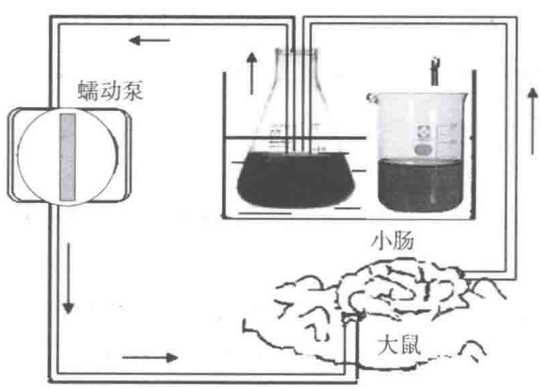


图 1　大鼠在体小肠吸收实验装置

（1）取100ml供试液（100ml Krobs－Ringer试液含磺胺嘧啶2mg、酚红2mg）加入循环装置的烧瓶中。

（2）将实验前禁食过夜（约18h），自由饮水，体重200g左右的雄性大鼠，称重，腹腔注射乌拉坦（剂量为100g体重注射0.4ml），麻醉后并加以固定。

（3）整个实验过程中开启红外灯，保持大鼠体温。沿腹中线打开腹腔（约3cm长）。自十二指肠上部及回肠下部各剪开一个小口，各插入直径为0.5cm的玻璃管，用线扎紧，并用37℃的生理盐水将小肠内容物冲洗干净，然后将大鼠串联到循环装置中。

（4）开动蠕动泵，以5ml/min的流速循环10分钟后流速调至2.5ml/min。

（5）自烧瓶中取样1.5ml（1ml、0.5ml各一份）为药物和酚红零时间样品，并补加2ml酚红溶液（每毫升Krobs－Ringer试液含酚红20μg）其后每15分钟取样（1ml、0.5ml各一份），同时补加酚红溶液。由于酚红不被小肠吸收，用以测定水被小肠吸收的量。

4. 定量方法

（1）磺胺嘧啶的定量　取样品1ml，加入1mol/L HCl 5ml，加入0.1% $NaNO_2$ 1ml，摇匀，放置3分钟，加入0.5%氨基磺酸铵1ml，摇匀，放置3分钟。加入0.1%萘乙胺2ml，摇匀，放置20分钟。在550nm处测定吸收度。

参比溶液的配制：取1ml酚红试液按磺胺嘧啶的定量方法配制。

（2）酚红定量　样品0.5ml，加入0.2mol/L NaOH 5ml，摇匀，在555nm测定吸收度。

参比溶液：酚红的比色空白液为0.2 mol/L NaOH。

5. 标准曲线的制备

（1）酚红的标准曲线　精密称取酚红10mg，置100ml容量瓶内，加1% Na_2CO_3 溶液溶解并稀释至刻度，制成100μg/ml的标准溶液。取1ml，2ml，3ml，4ml，5ml，6ml的标准溶液于10ml容量瓶中，加蒸馏水至刻度。自上述各溶液中吸取0.5ml，按酚红的定量方法测定吸收度，并绘制标准曲线。

（2）磺胺嘧啶的标准曲线

贮备液的配制：精密称取磺胺嘧啶标准品10mg，置100ml容量瓶中，以1% Na_2CO_3 溶液20ml溶解，以蒸馏水溶解并稀释至刻度，使成100μg/ml的标准溶液。

标准曲线制备：取上述贮备液适量，分别吸取1ml、2ml、3ml、4ml、5ml、6ml于10ml容量瓶中，加蒸馏水至刻度。从上述溶液中各吸取1ml按磺胺嘧啶定量方法测定吸收度，并绘制标准曲线 。

【实验结果与讨论】

将实验数据按表1中公式进行计算，以剩余药量的对数对时间作图，求出吸收速度常数 k_a，和每小时吸收率（%）。

$$\text{每小时吸收率（\%）} = \frac{\text{零时间剩余药量} - 60\text{分钟剩余药量}}{\text{零时间剩余药量}} \times 100\%$$

表1 大鼠在体小肠吸收量的计算式

取样时间（h）	磺胺嘧啶		酚红		供试液体积（ml）	剩余药量（μg）
	吸收度	浓度（μg/ml）	吸收度	浓度（μg/ml）		
循环前	Ao	C_0	A_0'	C_0'	$V_0 = 100\text{ml}$	$P_0 = 100 \times C_o$
0	A_1	C_1	A_1'	C_1'	$V_1 = \frac{C_0' V_0}{C_1'}$	$P_1 = C_1 V_1$
0.25	A_2	C_2	A_2'	C_2'	$V_2 = \frac{(V_1 - 1.5) \cdot C_1' + 40}{C_2'}$	$P_2 = C_2 V_2 + 1.5 C_1$
0.5	A_3	C_3	A_3'	C_3'	$V_3 = \frac{(V_2 - 1.5)\ C_2' + 40}{C_3'}$	$P_3 = C_3 V_3 + 1.5\ (C_1 + C_2)$
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
t_n	A_n	C_n	A_n'	C_n'	$V_n = \frac{(V_1 - 1.5)\ C_{n1}' + 40}{C_n'}$	$P_n = C_n V_n + 1.5 \sum_{i=1}^{n-1} C_i$

【思考题】

1. 做好本实验的关键是什么？在操作中应该注意哪些问题？
2. 本实验装置能否进一步改进？

Experiment 2: *In situ* intestinal absorption experiment in rats

Purpose

1. To learn how to conduct the *in situ* intestinal absorption experiment in rats.

2. To learn how to calculate the first – order absorption rate constant (k_a) and the absorption percentage per hour.

Introduction

Most drugs pass through biomembranes from the high concentration side to the low concentration side via passive diffusion. Passive diffusion can be described by the Fick' s Law (Eq. 1), in which the diffusion rate (dC/dt) is proportional to the concentration difference between the two sides of the cellular membrane.

$$-dC/dt = k_a \triangle C = k_a\ (C - C_b) \qquad (1)$$

where C is the concentration of drug in the gastrointestinal (GI) tract at t hour, C_b is the concentration of drug in blood and k_a is the absorption rate constant. The diffusion rate (dC/dt) can be influenced by the diffusion coefficient of the drug, the membrane thickness and area, and the drug membrane permeability.

Gastrointestinal absorption is a process where a drug diffuses from the gastrointestinal side

(absorption site) to the blood side. Since the drug entering the blood will be quickly distributed throughout the body, the drug concentration in blood will be very low compared with that at the absorption site. Therefore, the blood compartment can be considered to be under "sink condition" after oral administration of most drugs. This has resulted in a large concentration gradient across the GI membrane during the entire absorption process. That is, $C \gg C_b$, so $\Delta C \approx C$, and Equation (1) can be rewritten as:

$$-dC/dt = k_a C \quad (2)$$

Equation (2) is consistent with the standard first - order kinetics. The absorption of most drugs from the gastrointestinal tract follows first - order kinetics. On the other hand, the membrane diffusion rate can be described as the reduction in the amount of drug at the absorption site (dX_a/dt):

$$-dX_a/dt = k_a X \quad (3)$$

Integrating equation (3) and then transforming it into the corresponding logarithmic form:

$$\ln X_a = \ln X_a\ (0)\ - k_a t \quad (4)$$

where X_a is the amount of drug in the gastrointestinal tract at t howr, X_a (0) is the amount of drug in the gastrointestinal tract at $t = 0$, k_a is the drug absorption rate constant. Plotting ln X_a versus t gives a straight line with the slope equal to k_a.

Methods

1. Apparatus

Peristaltic pump; UV spectrophotometer; infrared light source; surgical scissors hemostat; latex tubing; beaker; fixing plate; electrically heated thermostatically - controlled water bath.

2. Reagents

(1) 0.1% $NaNO_2$ solution.

(2) 0.5% $NH_2SO_3NH_4$ solution.

(3) 0.1% Naphthalene ethylenediamine dihydrochloride solution, (these three reagents should be stored in a refrigerator after preparation) .

(4) 1 M HCl, (5) 0.2 M NaOH.

(5) Physiological sodium chloride solution.

(6) Krebs - Ringer solution (1000 ml solution contains 7.8 g NaCl, 0.35 g KCl, 0.37 g $CaCl_2$, 1.37 g $NaHCO_3$, 0.32 g NaH_2PO_4, 0.02 g $MgCl_2$, and 1.4 g glucose) .

(7) Urethane (20% g/g, intra - peritoneal injection 0.4 ml per 100 g rat for anesthesia),

(8) Sulfadiazine (SD) .

3. Procedures

(1) Add 100 ml of test solution (containing SD 2 mg and phenol red 2 mg in 100 ml Krebs - Ringer' s solution) to the beaker of the circulation system (Figure 2 - 1) .

(2) Male rats weighing about 200 g are fasted overnight (about 18 hours) with free access to water before the experiment. Rats are then anesthetized with an intraperitoneal injection