

**Studies on Rats Intestinal Absorption Calcium**

**September 1998**

**ViVitec Japan Division**

## CONTENTS

### Studies on Rats Intestinal Absorption Calcium

<b>i . Materials and Methods</b>	<b>1</b>
<b>1.Animal</b>	<b>1</b>
<b>2. Housing environment(Rats room)</b>	<b>1</b>
<b>3. Test environment (Laboratory)</b>	<b>1</b>
<b>4. Test instrument equipment and agents</b>	<b>2</b>
<b>5. Materials</b>	<b>2</b>
<b>6. Test groups</b>	<b>2</b>
<b>7.Methods</b>	<b>2</b>
<b>II . Results</b>	<b>3</b>

## **Studies on Rats Intestinal Absorption Calcium**

### **1. Materials and Methods**

#### **1. Animal**

##### **1) Species, strain, sex**

**Species: rat, strain: SD, sex: male**

##### **2) Rats' age at the beginning of the test**

**(designated rats' age when delivered)**

**7 weeks (200g)**

##### **3) Microbiological grade**

**SPF**

##### **4) Breeder**

**Crea Japan Co., Ltd.**

#### **2. Housing environment (Rats Room)**

**1) Temperature & moisture: 24#1 t, 5515%**

**2) Air conditioning: 70% air circulation (return-air system)**

**3) Lighting: 12 hours automatic-lighting cycle**

**(lighting from 8 a.m. to 8 p.m.)**

**4) Cage: A stainless steel bracket cage with an auto-flush cleaning unit**

**5) Feed: CA-1 (Nippon Crea Co., Ltd.), sterilized solid food for mice, rats and hamsters**

**6) Water supply: Filtered tap water supplied by an automatic water-supply unit**

#### **3. Test environment (Laboratory)**

**1) Temperature & moisture: 2611 t, 5515%**

**2) Air conditioning: 70% air circulation**

**(return-air system)**

## 7. Methods

1) Preparation of recirculation solution 2mg of test calcium were dissolved in 10 ml of the artificial gastric juice\*1. Half an hour later, this solution was added to 10 ml of the artificial intestinal juice\*2, the pH was adjusted to 6.5 and exactly 100ml was obtained for the recirculation solution.

**\*1: Preparation of the artificial gastric juice**

24.0ml of dilute hydrochloric acid and ion exchanged water were added to 2.0g of sodium chloride to make 1,000ml of solution (pH: about 1.2).

**\*2: Preparation of the artificial intestinal juice**

118ml of 0.2N sodium hydroxide solution and ion exchanged water were added to 250ml of 0.2M monobasic potassium phosphate solution to make 1000ml of solution (pH: about 6.8).

## 2) Absorption test by *in situ* recirculation method''''

### Technique

The rats were deprived of food for 24 hours and an abdominal median incision was performed under urethane (1.2g/kg). The choledoch proximal to the duodenum was tied to eliminate the effects of the bile, and the area from the pyloric part to the part 6 cm below was assigned for recirculation. L-tubes were set at the start and end points of the recirculation and ligated with sutures. Saline was preheated to 37 °C and poured into the

L-tube at the start point to clean the intestine. After cleaning, a light was placed close to the rats to prevent loss of body temperature.

### Recirculation

The recirculation solution prepared as described earlier was preheated. The Perista pump and the reservoir containing the recirculation solution and test materials between the cannulas and the small intestine were set to form a loop (connecting the small intestine, the cannula, the test solution, the Perista pump, the cannula and back to the small intestine), the solution was circulated from the start point proximal to the pyloric to the lower part at a speed of 1 ml/min while the Ca concentration of the solution (decreased amount of Ca) was successively measured. Solution samples for Ca measurement were collected immediately after recirculation and at 10, 20, 30, 40, 50 and 60 minutes after. 1 ml of sample was taken each time.

Table 1. Intestinal absorption of calcium in rat duodenum by recirculating method.

	Circulating time (min)						mean
	10	20	30	40	50	60	
(Lactate)							
UNICAL CLD	4.05±1.01	3.82±0.83	4.55±0.23	6.96±0.38	7.82±0.46	9.05±0.53	6.04±2.20
Calcium Lactate	0.47±0.81	2.32±0.81	2.32±0.81	3.25±0.85	4.19±0.03	5.12±0.83	2.95±1.63
(Citrate)							
UNICAL CTM	3.84±0.25	5.39±0.37	6.31±0.36	6.06±0.27	6.81±0.29	7.29±0.35	5.95±1.22
UNICAL CCM	3.25±1.61	4.79±1.38	5.82±1.12	5.83±1.22	5.99±1.32	6.25±1.45	5.32±1.35
Calcium Citrate	1.09±0.91	2.03±1.22	3.00±1.10	3.61±1.11	3.68±1.10	5.63±1.34	3.17±1.13
(Carbonate)							
UNICAL CCM-C	3.06±0.58	3.33±0.22	4.61±0.31	5.90±0.35	6.92±0.22	7.49±0.23	5.22±1.85
Calcium Carbonate	1.32±0.57	1.65±0.58	2.63±0.58	2.96±1.00	3.29±0.58	4.28±0.58	2.67±1.11

Each value (mean ± SD) represents percentage of total intestinal absorption of calcium (n=5)