

# Some Problems in Esophageal Reconstruction Regarding the Tissue Respiration of the Digestive Tract

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## INTRODUCTION

Surgery of the esophagus has made remarkable progress on account of the development of anesthesia, blood transfusion and fluid infusion and antibiotics therapy together with improvement in operative techniques. Recently, further progress is being made due to the introduction of microvascular anastomosis with the use of the blood vessel suturing apparatus. In our Department, an intense postoperative oxygen therapy has been introduced as a countermeasure against sutural insufficiency which frequently occurs when esophageal reconstruction, especially the antethoracic esophagogastrostomy is performed, in an attempt to prevent hypoxia of the pedunculated gastrointestinal tract to be used for the reconstruction of the esophagus. The method to reconstruct the blood circulation at the tip of the pedunculated gastrointestinal tube<sup>26)</sup> through anastomosis of small blood vessels<sup>16) 17)</sup> and the method to improve the blood circulation in the pedunculated gastric tube through periarterial sympathectomy of the nutritional artery have also been studied. Some of these methods were applied in clinical cases and further attempts were made to improve the operative technic and the pre- and postoperative treatment, leading to a considerable improvement in the operative result. On the other hand, fundamental studies were also carried out on the etiology of pulmonary complication or reflux esophagitis<sup>15) 28) 43) 48)</sup> following the operation for the reconstruction of the esophagus. It is readily expected that an essential role is played by the biological nature and property of the tissue itself of various parts of the digestive tract to be used in the reconstruction of the esophagus and the esophagus itself in these problems directly related to the reconstructive operation of the esophagus as well as the pathogenesis of the postoperative reflux esophagitis<sup>8) 15) 48)</sup> and function of the transplanted gastrointestinal tract at a remote stage<sup>23) 31) 40) 42) 45)</sup>. Considering these points, tissue respiration of various parts of the digestive tract was measured from various standpoints in an attempt to solve several problems in the operation for the reconstruction of the esophagus. The following results were obtained.

## CHAPTER 1 STUDY ON TISSUE RESPIRATION OF VARIOUS PARTS OF THE DIGESTIVE TRACT INCLUDING THE ESOPHAGUS

Although the pedunculated or free graft from various parts of the digestive tract such as stomach, small and large intestines have been used for the reconstruction of the esophagus, sutural insufficiency at the site of anastomosis was seen in considerable frequency

due to the circulatory disturbance or hypoxia of the transplanted segment. The incidence was reported to be 0-6.8% upon intrathoracic esophagogastrostomy by means of the KIRSCHNER-NAKAYAMA type of gastric tube, and 50-100% in the antethoracic esophagogastrostomy<sup>17)</sup>. The most important cause of such sutural insufficiency is the circulatory disturbance in the elevated gastrointestinal tract, especially at the portion near the site of anastomosis<sup>25)</sup>. Various other factors also have to be taken into consideration in the selection of the portion of gastrointestinal tract to be used for the reconstruction of the esophagus and a final conclusion has not yet been obtained. Considering this point, the author tried to determine the amount of tissue respiration of each portion of the gastrointestinal tract by means of WARBURG's manometric technique.

### I. Experimental method<sup>12) 39) 49)</sup>

#### 1) Instrument and apparatus

WARBURG's apparatus made by F company, modified by NISHINO in our Department, was used. This instrument consists of a glass manometer with the internal diameter of 1 mm and main, accessory and side chambers, to be used for the determination at the temperature of the thermostatic chamber of 37.5°C, amplitude of 5.5 cm, and the number of vibrating revolutions of 82 per minutes.

2) Experimental materials. Healthy adult mongrel dogs with body weight ranging from 6 to 10 kg were used. The animals were fasted for 12 to 24 hours before operation. Laparotomy and thoracotomy were performed under anesthesia through intravenous administration of 25 mg/kg Nembutal. From each part of the digestive tract, esophagus, stomach, duodenum etc., cylindrical segments of approximately 1 cm in width were resected, washed in KREBS-RINGER phosphate buffer solution of pH 7.4 to remove blood, and cut into slices of 1 cm<sup>2</sup> in square surface. Using a razor blade, this slice was cut into a thin long square-shaped tissue fragment with the limited thickness on a filter paper. This tissue fragment was used for the experiment after weighing. After measuring the tissue respiration, this tissue fragment was placed into a weighing bottle to be dried at 60°C for 12 hours.

The dry weight was measured with an electric ultrachemical balance with the accuracy of 1/10 mg. When the dry weight was out of the previously set range, the results were discarded.

#### 3) Method of determination

For the measurement of tissue respiration, the direct method of WARBURG was employed. One of the 12 manometers was used as the thermobarometer, while the remaining 11 were used for the main experiment. The vessel constant of the cone-shaped vessel used was calculated by the following formula :

$$KO_2 = \frac{VG \frac{273}{273+t} + VF \alpha}{10}$$

where VF = volume of the fluid in the vessel (cc)

VG = volume of the gas chamber (cc)

t = temperature of the thermostatic chamber (37.5°C)

$\alpha$  = BUNSEN's coefficient of O<sub>2</sub> against water at 37.5°C. (0.238)

In the determination, 4 cc of KREBS-RINGER-phosphate buffer solution of pH 7.4 (10 cc of 0.9 % NaCl, 4 cc of 11.5 % KCl, 3 cc of 1.22 % CaCl<sub>2</sub>, 1 cc of 2.11 % KH<sub>2</sub>PO<sub>4</sub>, 1 cc of 3.82 % Mg SO<sub>4</sub>·7 H<sub>2</sub>O, and 12 cc of 0.1 M Na<sub>2</sub>HPO<sub>4</sub>) and the tissue fragment were placed in the main chamber. In the accessory chamber 0.2 cc of 10 % KOH solution was placed to absorb the generated CO<sub>2</sub>. In order to increase the area of contact with the gas, 5-6 square filter papers, cut into the size which made it possible for them to float freely, were placed in the accessory chamber. In this instance the side chamber was not used, although 0.2 cc of 2 % glucose solution was placed within the side chamber when the influence of the added glucose was determined. As the solution for the occlusion of the manometer, BRODIE's solution stained with Evans blue was used. The vessel thus prepared was attached to the manometer, fixed to the above-mentioned WARBURG manometric apparatus and shaken for 10 minutes in a thermostatic chamber with the amplitude of 5 cm and 82 vibrations per minutes. The valve was closed after the vessel and the manometer reached the temperature equilibrium. After shaking another 10 minutes, the reading of the manometer was done and further readings were carried out every 10 minutes with the accuracy of 0.5 mm. When the thermobarometer showed a difference of more than 3 cm, the results were discarded. The calculation of QO<sub>2</sub> was done with the following formula :

$$QO_2 = \frac{60 \cdot h \cdot KO_2}{Wt}$$

where h=the depression of the fluid surface in mm during t minutes

w=dry weight of the tissue fragment (mg)

KO<sub>2</sub>=vessel constant

## II. Experimental results

The results of determination of tissue respiration in the whole layer slice of various parts of the digestive tract in the healthy dogs in KREBS-RINGER phosphate buffer solution was as follows :

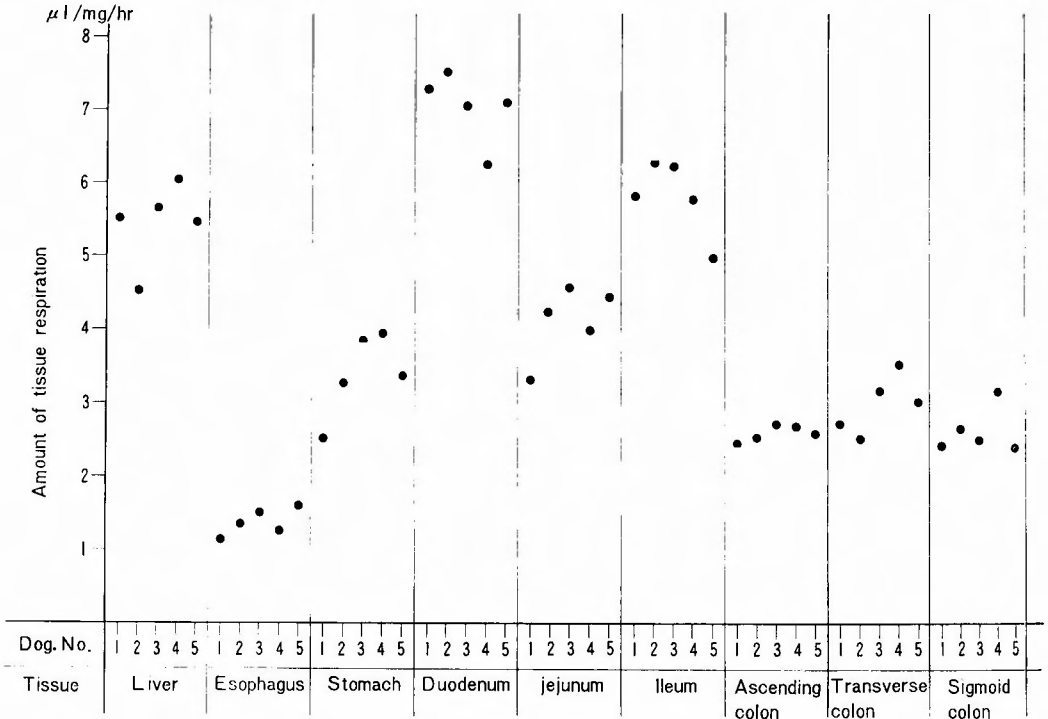
The amount of tissue respiration per hour was found to decrease gradually in the duodenum, ileum, liver, jejunum, stomach, ascending colon, transverse colon, sigmoid colon, and esophagus, in the order mentioned, as seen in Fig. 1. Although no great difference was noted between the mid-thoracic and lower thoracic esophagus, the pyloric region showed a slightly higher value than the cardiac region. As shown in Table 3, the tissue respiration of human esophagus was markedly lower than that of the stomach, when these tissues were obtained during the operation for cancer of the esophagus. Determinations in time course with 10 minutes' interval revealed no remarkable fluctuation of tissue respiration in various parts of the digestive tract (Table 4). On the other hand, the addition of 2 % glucose solution into the reaction mixture resulted in

**Table 1** Amount of tissue respiration in various parts of digestive tract in dogs. ( $\mu$ l/mg/hr)

Tissue	Dog No.				
	1	2	3	4	5
Liver	5.51	4.52	5.66	6.02	5.45
Esophagus	1.13	1.35	1.50	1.25	1.60
Stomach	2.50	3.25	3.84	2.94	3.36
Duodenum	7.26	7.50	7.02	6.23	7.07
Jejunum	3.30	4.21	4.55	3.98	4.43
Ileum	5.80	6.25	6.20	5.75	4.95
Ascending colon	2.44	2.51	2.70	2.66	2.57
Transverse colon	2.70	2.50	3.15	3.51	3.00
Sigmoid colon	2.41	2.65	2.48	3.15	2.39

**Table 2** Amount of tissue respiration in various portions of esophagus and stomach in dogs. ( $\mu\text{l}/\text{mg}/\text{hr}$ )

Dog No.	Tissue	Liver	Mid-thoracic esophagus	Abdominal esophagus	Stomach (pyloric region)	Stomach (cardiac region)
6		2.8	1.20	1.55	2.50	1.15
7		2.56	1.01	0.14	2.34	2.30
12		4.52	1.57	1.33	2.80	2.44
23		5.60	1.82	1.43	3.36	3.36
24		3.50	1.30	1.07	3.40	2.70



**Fig. 1** Amount of tissue respiration in various parts of digestive tract in dogs.

**Table 3** Amount of tissue respiration in the specimens of esophagus and stomach in patients suffering from cancer of the esophagus. ( $\mu\text{l}/\text{mg}/\text{hr}$ )

Clinical cases	Tissue	Liver	Stomach	Thoracic esophagus	Abdominal esophagus
Cancer of mid-thoracic esophagus (59-year-old male)		2.30	1.14	1.38	1.12
" (55-year-old male)			1.87	1.25	0.96
" (65-year-old male)		4.09	3.75	1.74	1.37
Cancer of cardia (58-year-old male)			1.93		1.08

a marked increase in the tissue respiration of the liver, while the rate of increase in the esophagus and stomach was slightly smaller (Table 5).

**CHAPTER 2 THE EFFECT OF BILE AND BILIARY ACID ON THE TISSUE RESPIRATION OF THE ESOPHAGUS, STOMACH, DUODENUM, JEJUNUM AND COLON**

As the cause of the sutural insufficiency at the site of anastomosis and postoperative reflux esophagitis, i.e. the complication of esophagogastrostomy, esophagojejunostomy and

**Table 4** Change of tissue respiration in various parts of digestive tract with the progress of time (Mean values in 5 healthy dogs). ( $\mu\text{l}/\text{mg}/\text{hr}$ )

Tissue \ Time min.	10'	20'	30'	40'	50'	60'
Liver	0.90	1.22	1.25	0.40	1.02	0.05
Stomach (pyloric region)	0.25	0.40	0.74	0.46	0.45	0.34
Stomach (cardiac region)	0.20	0.26	0.50	0.24	0.06	0.21
Mid-thoracic esophagus	0.15	0.23	0.45	0.20	0.45	0.19
Lower thoracic esophagus	0.13	0.22	0.20	0.33	0.42	0.17
Jejunum	0.7	1.06	1.35	1.02	1.01	0.44
Sigmoid colon	0.27	0.39	0.55	0.48	0.42	0.29

**Table 5** Change of tissue respiration in the esophagus, stomach and liver of dogs by adding 0.2 cc of 2% glucose solution into the reaction mixtures. ( $\mu\text{l}/\text{mg}$ )

Tissue \ Glucose \ Time min.	10'	20'	30'	40'	50'	60'	
Liver	-	0.63	1.24	1.61	2.15	2.74	3.12
	+	0.65	2.01	3.39	4.86	5.88	6.90
Rate of increase		3%	62%	111%	126%	115%	121%
Stomach	-	0.60	1.04	1.27	1.48	2.63	3.00
	+	0.81	1.46	1.46	2.52	3.22	3.60
Rate of increase		35%	40%	15%	70%	22%	20%
Esophagus	-	0.34	0.84	1.12	1.15	1.26	1.39
	+	0.50	1.53	1.04	1.80	2.16	1.77
Rate of increase		47%	82%	46%	57%	11%	27%

esophagocolostomy, participation of bile has been suggested. The following experiments were conducted to study the influence of bile and biliary acid on the tissue respiration of the esophagus, stomach, jejunum and sigmoid colon.

#### I. Experimental method

##### 1) Experimental instrument and apparatus

The same instrument and apparatus as described in Chapter 1 were used.

##### 2) Experimental materials

Tissue segments were resected from various parts of the digestive tracts of healthy mongrel dogs with body weight ranging from 6 to 10 kg with the method described in Chapter 1. Limit slices were similarly prepared.

##### 3) Drugs used

###### a) Bile

Bile was obtained aseptically from the gallbladder of the experimental dog, placed in a sterilized test tube, adjusted to pH 7.4, and 0.2 cc aliquot was placed in the side chamber. It was added into the reaction mixture during the experiment.

###### b) Bile acid solution

In 100 cc of distilled water, 0.2 g of bile acid, sodium taurocholate (Nutritional Biochemical Corporation, USA) was dissolved. The pH was adjusted to 7.4 and the solution was kept in a colored bottle. The 0.2 cc aliquot was used when necessary.

## 4) Method of measurement

The limit tissue fragment was floated in the main chamber of the apparatus containing 4.0 cc of KREBS-RINGER phosphate buffer solution. An amount of 0.2 cc of 10 % KOH was placed with small filter paper fragments in the accessory chamber and 0.2 cc of bile or bile acid solution prepared according to the method stated previously was placed in the side chamber. The vessel was fixed to the thermostatic chamber at 37.5°C together with the manometer, shaken for 10 minutes. After the temperature equilibrium between the vessel and the manometer was reached, the stop-cock was closed and the bile or bile acid solution in the side chamber was transferred into the main chamber. The pressure measurement was conducted every 10 minutes for 60 minutes.

## II. Experimental results

As shown in Tables 6 and 7, bile and bile acid markedly inhibited the tissue respiration of the stomach, esophagus, sigmoid colon, jejunum, and the liver, in this order, and the inhibition was more pronounced in bile acid than in bile at the concentration used.

**Table 6** Effect of bile on tissue respiration in various parts of digestive tract. ( $\mu\text{l}/\text{mg}/\text{hr}$ )

Dog No.	Bile	Tissue	Esophagus	Stomach	Jejunum	Sigmoid colon	Liver
16	—		1.35	2.52	4.20	2.21	5.41
	+		1.09	1.75	3.64	1.73	5.39
17	—		1.50	3.73	4.54	2.70	5.65
	+		1.24	1.85	4.15	2.50	4.84
25	—		1.60	3.25	3.93	2.49	5.86
	+		1.33	1.44	3.85	1.97	4.64
Inhibitory rate		(%)	17.3	4.6	8.3	12.5	7.5

**Table 7** Effect of biliary acid on tissue respiration in various parts of digestive tract. ( $\mu\text{l}/\text{mg}/\text{hr}$ )

Dog No.	Sodium taurocholate	Tissue	Esophagus	Stomach	Jejunum	Sigmoid colon	Liver
26	—		1.15	2.50	3.35	2.17	4.16
	+		1.32	2.51	3.34	2.41	4.07
27	—		1.35	3.23	4.11	2.75	5.70
	+		0.87	2.12	4.45	1.44	5.21
29	—		1.50	3.85	4.50	2.50	5.14
	+		1.05	2.37	4.37	2.10	5.44
31	—		1.24	2.86	3.93	3.15	4.65
	+		0.03	2.88	3.91	2.32	4.01
32	—		1.72	3.35	4.40	2.34	5.84
	+		1.13	2.03	4.07	2.60	4.05
Inhibitory rate		(%)	22.2	26.1	1.2	23.0	14.0

### CHAPTER 3 COMPARATIVE STUDIES ON THE FUNCTION OF THE GASTRIC TUBES USED FOR ANTETHORACIC AND INTRATHORACIC RECONSTRUCTION OF THE ESOPHAGUS FROM THE ASPECT OF TISSUE RESPIRATION

For the reconstruction of the esophagus, the KIRSCHNER-NAKAYAMA type of gastric tube is most frequently used at present. However, the function of these gastric tubes at a remote date has been studied from the standpoint of motility and secretion only on 2 or 3 occasions<sup>23) 40) 46) 48)</sup>. For the purpose of studying the influence of the environment of transplantation on the function of the gastric tube, the following experiments were conducted.

#### I. Experimental method

##### 1) Experimental material

Healthy adult mongrel dogs with body weight of approximately 10 kg were fasted for 12 to 24 hours before the operation. Under intravenous anesthesia with 25 mg/kg Nembutal while keeping the airway and oxygen inhalation in a closed system with intratracheal apparatus for anesthesia, laparotomy and subsequently thoracotomy were carried out. The pedunculated gastric tube was prepared according to the KIRSCHNER and NAKAYAMA method.

The stump was made into a blind end and elevated antethoracically or into the thorax cavity. The gastric tube was fixed subcutaneously in front of the chest cavity when the antethoracic technique was used. On the other hand, when intrathoracic transplantation was used, the diaphragm was incised slightly at the left side of the hiatus esophageus. From the opening of this incision the gastric tube was elevated into the thorax cavity, taking care not to interfere with the movement of the lung and heart, and fixed to the edge of the diaphragmatic incision. The gastric remnant in the abdominal cavity was anastomosed to the jejunum in the fashion of the antecolic gastrojejunostomy. The animal was then fed for 3 months, taking care not to cause nutritional disturbance. About 3 months after the above-mentioned operative procedure, segments were resected from the elevated pedunculated gastric tube, gastric remnant in the abdominal cavity and thoracic esophagus in the surviving animal, to prepare the limit slice of each.

##### 2) Method of determination

In the constant amount of limit slice of each tissue prepared according to the method described above, tissue respiration was measured with the method described in Chapter 1.

#### II. Experimental results

As shown in Table 8, the tissue respiration of the elevated pedunculated gastric tube was lower than the gastric tissue without manipulation in a follow-up study. In comparing the tissue respiration between intrathoracically and antethoracically transplanted gastric tubes and the gastric remnant in the abdominal cavity, the former two gave a slightly lower value. The intrathoracically transplanted gastric tube showed a lower tissue respiration than the antethoracically transplanted gastric tube. The tissue respiration of the esophagus did not show any difference between the operated and non-operated groups.

**Table 8** Comparison of amounts of tissue respiration among the esophagus, ante- and intrathoracically transplanted gastric tube and abdominal gastric remnant. ( $\mu\text{l}/\text{mg}/\text{hr}$ )

Dog No.	Tissue	Esophagus	Antethoracically transplanted stomach	Intrathoracically transplanted stomach	Abdominal gastric remnant	Days after the operation
22		1.21	2.16 (1.78)		2.57	120
23		1.33	2.05 (1.54)		2.40	90
30		0.98		2.02 (2.06)	2.51	34
52		1.67	2.51 (2.34)		2.78	105
57		1.48		2.11 (1.43)	3.03	29
61		1.56	3.17 (2.04)		3.77	126
63		1.50		2.76 (1.80)	2.20 (1.93)	33

Numbers in the brackets show the amended values, on the assumption that the value of esophageal tissue respiration is constant.

#### CHAPTER 4 INFLUENCE OF CYTOCHROME C, VITAMIN B<sub>2</sub> IN ACTIVE FORM AND NICOTINIC ACID AMIDE ON THE TISSUE RESPIRATION OF VARIOUS PARTS OF THE DIGESTIVE TRACT BEGINNING WITH THE ESOPHAGUS

Cytochrome C which has recently been applied in the treatment of various vascular disturbances is expected to alleviate the effect of circulatory disturbance in the gastrointestinal segment used for the reconstruction of the esophagus by activating the oxygen brought to body cells and facilitating the cell respiration. On the other hand, the combined use of nicotinic acid amide and activated vitamin B<sub>2</sub>, or FAD, coenzymes of dehydrogenase and yellow enzyme, carrying the activated hydrogen from the TCA cycle, would especially augment the effect of cytochrome C administration. For the purpose of clarifying these points, the following fundamental experiments were attempted.

##### I. Experimental method

##### 1) Experimental material

Healthy adult mongrel dogs were used. The limit slices were prepared from the esophagus, stomach and the liver with the method described in Chapter 1.

##### 2) Drugs used

Those used for the manometric measurement are described in Chapter 1. Cytochrome C was mainly given in the form of cytomack (3 mg/cc, Nippon Shin-yaku Co., Ltd.) and partially in the form of cytochron (4 mg/cc, Sankyo Co. Ltd.), active Vitamin B<sub>2</sub> was given in the form of Flavitan (5-10 mg/cc, Yamanouchi Co. Ltd.) and nicotinic acid amide was given in the form of Niamide (10 mg/cc, Sonnebod Co. Ltd.). The drugs were diluted with KREBS-RINGER phosphate buffer solution to the necessary dilution when required.

##### 3) Method of determination

The method described in Chapter 1 was used to prepare the whole layer limit slices from the esophagus, stomach and the liver. A constant amount of the slice was floated in the main chamber containing 4.0 cc of KREBS-RINGER phosphate buffer solution. In the accessory chamber, few small pieces of filter paper were floated in 0.2 cc of 10% KOH. In the side chamber, 0.4 cc of various drugs were placed. Experiments were conducted in the following 4 groups according to the kinds of drugs used. (1) In the control group,



nothing but KREBS-RINGER phosphate buffer solution was added, (2) 1.2 mg of cytochrome C alone was added, (3) 2 mg of active vitamin B<sub>2</sub> and 2 mg of nicotinic acid amide were added, (4) 1.2 mg of cytochrome C, 2 mg of active vitamin B<sub>2</sub> and 2 mg of nicotinic acid amide were added. The WARBURG manometer was used to measure the tissue respiration as described in Chapter 1. When the volume exceeded the size of the chamber as in group 4, the fluid in the main chamber was decreased and the fluid in the accessory chamber was added to the main chamber.

II. Experimental results

1) Addition of cytochrome C alone

As shown in Table 9, the tissue respiration of the esophagus, stomach and the liver surpassed the level in the control group. The rate of activation was the highest in the esophagus, followed by the stomach and the liver, indicating a higher value in organs with muscular layers.

**Table 9** Effect of cytochrome c on tissue respiration in various parts of digestive tract. ( $\mu\text{l}/\text{mg}/\text{hr}$ )

Dog No.	Tissue	Liver		Esophagus		Stomach	
	Cytochrome C 2mg	added	none	added	none	added	none
33		1.60	1.30	1.28	0.25	0.30	0.17
34		2.51	2.24	2.00	0.62	1.35	0.25
41		3.43	2.62	1.81	0.27	0.76	0.21
42		3.05	2.37	2.43	0.32	1.42	0.33
43		3.46	1.59	2.50	0.61	1.75	0.46
Rate of activation (%)		39.1		355		292	

2) Addition of active vitamin B<sub>2</sub> and nicotinic acid amide

As shown in Table 10, the tissue respiration was higher in the esophagus, stomach and the liver as compared with the controls. The rate of activation was found to be high in the stomach, esophagus and liver in this order, and was greater than that with the addition of cytochrome C alone, especially in the liver.

**Table 10** Effect of active vitamin B<sub>2</sub> combined with nicotinic acid amide on tissue respiration in various parts of digestive tract. ( $\mu\text{l}/\text{mg}/\text{hr}$ )

Dog No.	Tissue	Liver		Esophagus		Stomach	
	Vitamin B <sub>2</sub> , Nicotinic acid amide	added	none	added	none	added	none
34		4.30	2.24	1.90	0.62	1.30	0.17
41		5.02	2.62	2.01	0.27	0.96	0.25
42		4.94	2.37	2.50	0.32	1.77	0.21
43		5.87	1.59	2.33	0.61	1.55	0.46
Rate of activation (%)		127		384		415	

3) Addition of cytochrome C, active vitamin B<sub>2</sub> and nicotinic acid amide

As shown in Table 11, a marked increase in tissue respiration was seen in the esophagus,

phagus, stomach and liver as compared with the controls. The rate of activation was respectively high in the stomach, esophagus and liver, in this order, especially high in the esophagus and the stomach as compared with the results in (1) and (2).

**Table 11** Effect of cytochrome c combined with active vitamin B<sub>2</sub> and nicotinic acid amide in tissue respiration in various parts of digestive tract. ( $\mu\text{l}/\text{mg}/\text{hr}$ )

Dog No.	Tissue Cytochrome C, Vitamin B <sub>2</sub> , Nicotinic acid amide	Liver		Esophagus		Stomach	
		added	none	added	none	added	none
33		4.35	1.30	1.33	0.25	1.20	0.17
34		4.61	2.24	2.65	0.62	2.71	0.25
41		5.05	2.62	2.57	0.27	1.96	0.21
42		5.50	2.37	2.78	0.32	2.43	0.33
43		5.72	1.57	3.04	0.61	2.80	0.46
Rate of activation (%)		140		502.5		692	

4) Tables 12 and 13 show changes in terms of time course in tissue respiration upon addition of cytochrome C, active vitamin B<sub>2</sub> and nicotinic acid amide to each tissue of the esophagus and the stomach.

**Table 12** Change of tissue respiration in the esophagus and stomach by adding 2 mg of cytochrome c into the reaction mixtures. ( $\mu\text{l}/\text{mg}$ )

Tissue	Time min.	Time					
		10'	20'	30'	40'	50'	60'
Control (stomach)		0.35	0.56	1.01	1.26	1.28	1.55
Stomach I		0.36	0.70	1.22	1.72	2.18	2.26
Stomach II		0.73	2.58	1.71	6.75	7.79	8.70
Esophagus I		0.15	0.37	0.45	0.68	0.50	0.46
Esophagus II		0.55	1.52	2.00	2.48	2.78	2.65

**Table 13** Change of tissue respiration in the esophagus and stomach in the same way as in the former experiment by adding cytochrome c together with active vitamin B<sub>2</sub> and nicotinic acid amide. ( $\mu\text{l}/\text{mg}$ )

Tissue	Time min.	Time					
		10'	20'	30'	40'	50'	60'
Control (stomach)		0.35	0.56	1.01	1.26	1.28	1.55
Stomach I		1.20	2.74	3.02	3.74	4.27	4.56
Stomach II		1.20	3.33	5.01	6.14	1.85	7.26
Esophagus I		0.62	0.90	1.95	1.38	0.84	0.38
Esophagus II		1.08	2.57	3.80	1.75	6.02	6.66
Esophagus III		2.00	4.21	5.44	6.56	7.81	9.23

#### CHAPTER 5 EFFECT OF ADMINISTRATION OF CYTOCHROME C, VITAMIN B<sub>2</sub> AND NICOTINIC ACID AMIDE ON THE CIRCULATORY DISTURBANCE OF THE DIGESTIVE TRACT

The results of in vitro experiment in Chapter 4 demonstrated a marked activation of tissue respiration upon addition of cytochrome C, active vitamin B<sub>2</sub> and nicotinic acid amide to various parts of the digestive tract, resulting in the efficiency of utilization of oxygen. Since a considerable alleviation of the effect of circulatory disturbance in the pedunculated gastrointestinal segment is expected upon administration of cytochrome C,

and the combined use of cytochrome C, nicotinic acid amide and active vitamin B<sub>2</sub>, the following *in vivo* experiments were carried out.

#### I. Experimental method<sup>42)</sup>

1) The method of preparation of the ROUX type of pedunculated jejunal loop. Healthy adult mongrel dogs of approximately 10 kg were fasted for 12 to 24 hours preoperatively. Laparotomy was performed under intravenous anesthesia with 25 mg/kg Nembutal. The region of the jejunum which was 40-50 cm off to the anal side from the TREITZ's ligament was pulled out of the wound, and the 3rd and 4th primary mesenteric vessels counted from the TREITZ's ligament were tied and severed. The jejunum was then amputated at 10-20cm from the TREITZ's ligament. At this place the secondary and tertiary branches of mesenteric arteries and veins were tied and severed to prepare the ROUX's pedunculated jejunal loop. Jejunal loops thus prepared had a closed tip. Marks placed with a silk thread at 1.5 cm interval from the base of the pedunculated jejunum, which was entirely free of circulatory disturbance, towards the tip.

2) Method of preparation of <sup>32</sup>P labeled erythrocytes suspension<sup>25)27)</sup>. The method used in the measurement of circulating blood volume was employed. To 20 cc of blood obtained from the femoral vein of the experimental dog were added 1 cc of 10% sodium citrate solution and 15 cc of physiological saline solution. The mixture was centrifuged at 2,000 rpm for 10 minutes. A similar procedure was repeated 3 times and <sup>32</sup>P orthophosphate was added to the red cell mass obtained, followed by physiological saline solution to make up the total volume of 20 cc. After thorough mixing, the material was incubated for 2 hours at 37°C. The vessel was gently shaken twice during the incubation. A similar procedure was repeated three times thereafter for washing to prepare 20 cc of <sup>32</sup>P labeled red cell suspension in physiological saline solution.

3) Method of determination. The ROUX's pedunculated jejunal loop was elevated antethoracically. After inserting a thin lead plate between this and the chest wall, 20 cc of <sup>32</sup>P labeled red cell suspension was injected into the femoral vein of the experimental dogs.

A Geiger-Müller counter produced by the Shimazu Company prepared into the area for the measurement of 2 mm in diameter was used to count the radioactivity at each marked site from the base of the pedunculated jejunal loop at 1.5 cm intervals. Natural count was deduced to calculate the relative radioactivities (cpm). Similar counting was conducted at one point in the peripheral portion of the jejunum without any operative treatment at all as the control for the purpose of correcting against the decline of the radioactivity in the circulating blood during the determination. MAJIMA of our Department<sup>25)</sup> studying the time course of changes in the radioactivity in circulating blood following the injection of <sup>32</sup>P labeled red blood cell suspension in physiological saline solution stated that the determination should preferably be started at a relatively stable period after the sudden increase upon injection and a rapid decrease of radioactivity or approximately 10 minutes after the injection, and finished within 60 minutes. These points were taken into consideration in the present experiment. Thus, the volume of circulating blood in each site of the jejunal loop, immediately after being prepared, were represented as the percentage of the normal. After the measurement of radioactivity was finished, the lead plate under the elevated jejunal loop was removed, the jejunal loop fixed antethoracically

and covered with the skin. After 48 hours, the wound in the anterior chest was opened under intravenous anesthesia to observe the changes in the color tone or occurrence of necrosis of the elevated pedunculated jejunal loop on account of circulatory disturbance. The spots were divided into those with an intense congestion without cyanosis, those appearing dark red to the addition of cyanosis to congestion, and those appearing black due to necrosis. The borderline of the latter two was treated as the limit of resistance to necrosis. With these methods as the fundamental approach, the drugs described in chapter 4 were used in the following 3 groups:

(1) No injection of drugs. (2) 1.5 mg/kg/day of cytochrome C was injected intravenously. (3) 1.5 mg/kg/day of cytochrome C, 1.5 mg of active vitamin B<sub>2</sub> and 1.5 mg of nicotinic acid amide were injected intravenously. The state of blood circulation in the elevated pedunculated jejunal loop 48 hours later was observed. By comparing the volume of circulating blood in the borderline of necrosis in the pedunculated jejunal loop, the effect of each preparation to improve the blood circulation was studied. In each group, 3 intravenous administrations were given in total from the day of operation to reoperation.

II. Experimental results

As shown in Tables 14, 15, 16 and 17, the volume of circulating blood through the borderline area of necrosis was less than 57.3% of the normal in the control group but

**Table 14** Effect of cytochrome c combined with active vitamin B<sub>2</sub> and nicotinic acid amide on the disturbance of blood circulation in the pedunculated jejunal loop.

(1) Control group

Dog No.15, N.C. 44cpm, Normal control portion 216cpm

Site of measurement	Value of measurement (cpm)	%	Color of jejunal loop
0	229		
1	180	78.6	
2	159	69.4	
3	154	67.3	
4	162	70.7	
5	144	62.9	reddish
6	131	57.3	dark red
7	101	(44.1)	necrotic
8	106	(46.3)	
9	115	(50.2)	
10	111	(48.5)	
11	103	(45.0)	
12	115	(50.2)	
13	118	(51.5)	
14	99	(43.2)	

The necrosed sites are marked by ( ), and radioactivities at all these sites were below 57.3% of the normal.

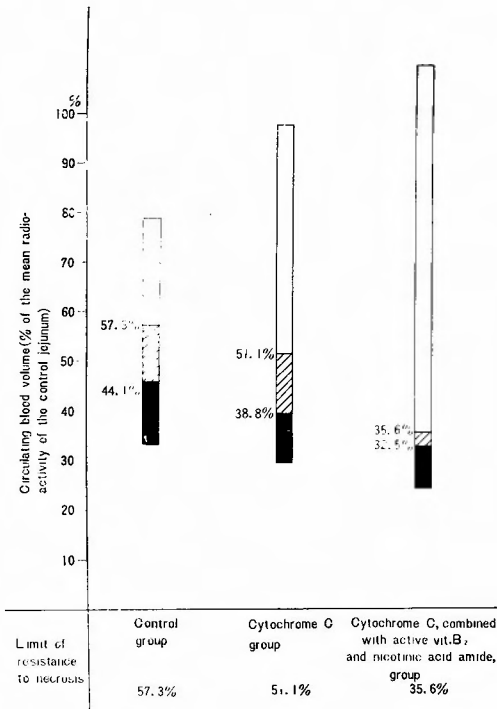
**Table 15** Effect of cytochrome c combined with active vitamin B<sub>2</sub> and nicotinic acid amide on the disturbance of blood circulation in the pedunculated jejunal loop.

(2) Cytochrome c group

Dog No.18, N.C. 45cpm, Normal control portion 241cpm

Site of measurement	Value of measurement (cpm)	%	Color of jejunal loop
0	276		
1	269	97.5	
2	215	77.9	
3	228	82.6	
4	209	75.7	
5	214	77.5	
6	197	71.4	
7	212	76.8	reddish
8	153	55.4	dark red
9	141	51.1	
10	104	(37.7)	necrotic
11	107	(38.8)	
12	107	(38.8)	
13	80	(28.9)	
14	84	(32.6)	
15	93	(33.7)	

The necrosed sites are marked by ( ), and radioactivities at all these sites were below 51.1% of the normal.



**Fig. 2** Effect of cytochrome c combined with active vitamin B<sub>2</sub> and nicotinic acid amide, on the disturbance of blood circulation in the pedunculated jejunal loop.

**Table 17** Effect of cytochrome c combined with active vitamin B<sub>2</sub> and nicotinic acid amide on the disturbance of blood circulation in the pedunculated jejunal loop.

(3) Cytochrome c combined with active vitamin B<sub>2</sub> and nicotinic acid amide group

Dog No. 21, N.C. 110cpm, Normal control portion 3962 cpm

Site of measurement	Value of measurement (cpm)	%	Color of jejunal loop
0	4465		
1	4755	106.5	
2	3689	82.6	
3	3589	80.4	
4	3513	78.5	
5	4066	91.1	
6	3363	75.3	
7	2900	64.9	
8	2288	51.2	
9	1075	24.1	
10	2028	45.4	
11	1602	35.9	
12	1580	35.6	
13	1154	25.8	reddish
14	1332	29.8	dark red
15	1174	(26.3)	dark red
16	1295	(29.0)	necrotic
17	1451	(32.5)	
18	1312	(29.4)	

The necrosed sites are marked by ( ), and radioactivities at all these sites were below 29.8-35.6% of the normal.

**Table 16** Effect of cytochrome c combined with active vitamin B<sub>2</sub> and nicotinic acid amide on the disturbance of blood circulation in the pedunculated jejunal loop.

(3) Cytochrome c combined with active vitamin B<sub>2</sub> and nicotinic acid amide group

Dog No. 20, N.C.45cpm, Normal control portion 1909 cpm

Site of measurement	Value of measurement (cpm)	%	Color of jejunal loop
0	1235		
1	2176	93.7	
2	2571	116.6	
3	2500	113.4	
4	2195	99.6	
5	2164	98.1	
6	2083	94.5	
7	2119	96.1	
8	2106	95.5	
9	1626	73.7	
10	1709	77.5	
11	1200	54.2	
12	1229	55.7	reddish
13	856	38.8	
14	928	42.1	dark red
15	882	(40.0)	necrotic
16	808	(36.6)	

The necrosed sites are marked by ( ), and radioactivities at all these sites were below 42.1% of the normal.

less than 51.1 % in the group treated with cytochrome C, giving a slightly lower value. The corresponding value in the group treated by cytochrome C, active vitamin B<sub>2</sub> and nicotinic acid amide was less than 29.8-42.1%, representing a markedly low value. The treatment with cytochrome C alone was able to reduce the influence of the circulatory disturbance in ROUX's pedunculated jejunal loop considerably, while the use of active vitamin B<sub>2</sub> and nicotinic acid amide together with cytochrome C augmented the effect (Fig. 2).

**CHAPTER 6 EFFECT OF STORAGE AT LOW TEMPERATURE AND PERFUSION  
ON THE TISSUE RESPIRATION OF THE SEGMENT OF THE DIGESTIVE  
TRACT USED IN COMPLETE FREE TRANSPLANTATION**

Recently, the complete free transplantation of the graft of the digestive tract to replace the defect of the esophagus has been performed in an increased frequency due to the progress in vascular surgery. The storage of the graft at low temperature is an important problem in homo-, hetero-, and auto-transplantation in two-stage operation. The free transplants of the stomach and sigmoid colon were experimentally studied concerning the changes in tissue respiration before and after storage at a low temperature. Since opinions have been divided concerning the use of perfusion immediately after removal of the free transplant to wash out thrombus and residual blood, free transplants from the sigmoid colon were studied concerning the tissue respiration with and without perfusion immediately after removal. Effect of storage at a low temperature after perfusion was also studied.

I. Experimental method

1) Method of preparation of free transplant from the stomach and sigmoid colon. Healthy adult mongrel dogs of approximately 10kg body weight were laparotomized under intravenous anesthesia with 25 mg/kg of Nembutal.

From the greater curvature side of the stomach, a segment of gastric tube approximately 10cm in length including gastroepiploic vessels was removed according to HEIMLICH's method. On the other hand, approximately 10 cm of the sigmoid segment including the sigmoid vessels was obtained and blood was removed from each transplant.

2) Method of storage at a low temperature<sup>18)</sup>

The transplants prepared as described previously were kept in an ice-chamber at 4°C for 48 hours. For the purpose of preventing the drying of the transplants, the transplants were placed in a vessel containing a small amount of physiological saline solution. The vessel was then covered with a gauze which had been dipped into physiological saline solution.

3) Method of perfusion

A perfusate was prepared with 200 cc of 5% low molecular weight dextran solution containing 32 mg of heparin and 5 cc of 4% xylocaine. The transplant was perfused until it became completely white in color in a dripping fashion under approximately 1.5m hydrostatic pressure, off and on. A perfusate containing an additional 12.5 mg of chlorpromazine in the above solution was then prepared<sup>9)</sup> and perfusion was similarly carried out. The tissue respiration of each free transplant was then determined. Furthermore, part of the transplant thus perfused was stored in a 4°C ice-chamber for 48 hours and the tissue respiration was determined.

4) Method of measuring tissue respiration

The limit slice was prepared from the transplant which was stored at a low temperature or subjected to perfusion and the tissue respiration of each fragment was determined using WARBURG manometer according to the method of determination described in Chapter 1.

II. Experimental results

As shown in Table 18, the tissue respiration of the gastric free graft was decreased by 48.3 % when only blood was removed from the transplant and the tissue was stored at 4°C for 48 hours. As shown in Table 19, on the other hand, sigmoid transplant only showed a decrease by 4.9 %. As seen in Table 20, upon perfusion of the sigmoid transplants with a perfusate containing 5 % low molecular weight dextran with the addition of 32 mg heparin and 5 cc of 4 % xylocaine, the decrease of tissue respiration due to perfusion was 31.4%. As shown in Table 21, the addition of 12.5mg of chlorpromazine to the perfusate resulted in a decrease of tissue respiration by 27.9%. The rate of decrease in tissue respiration was, therefore, small with the addition of chlorpromazine. As shown in Table 22, storage of sigmoid transplant at 4°C for 48 hours after the perfusion resulted in a decrease of tissue respiration by 65.0 %, representing an extreme decrease. The addition of chlorpromazine to the perfusate, moreover, could not prevent such decrease.

**Table 18** Effect of storage at a low temperature on tissue respiration of the gastric free graft.

( $\mu\text{l}/\text{mg}/\text{hr}$ )		
Dog No.	Before storage	After storage
48	3.25	1.25
49	3.31	0.25
52	2.88	1.76
53	3.92	2.17
Mean rate of decrease (%)	48.3	
Preserved at 4°C for 48 hrs.		

**Table 19** Effect of storage at a low temperature on tissue respiration of the free graft of sigmoid colon.

( $\mu\text{l}/\text{mg}/\text{hr}$ )		
Dog No.	Before storage	After storage
48	2.20	3.35
49	2.65	1.90
52	2.50	2.98
53	3.17	2.21
59	2.64	2.08
Mean rate of decrease (%)	4.9	
Preserved at 4°C for 48 hrs.		

**Table 20** Effect of perfusion on tissue respiration of the gastric free graft.

Perfusate : 5% low molecular weight dextran solution, added with heparin and xylocaine.

( $\mu\text{l}/\text{mg}/\text{hr}$ )		
Dog No.	Before perfusion	After perfusion
65	2.15	1.73
66	2.51	1.36
68	2.32	1.09
Mean rate of decrease (%)	31.4	

**Table 21** Effect of perfusion on tissue respiration of the free graft of sigmoid colon.

Perfusate : 5% low molecular weight dextran solution, added with heparin, xylocaine and chlorpromazine.

( $\mu\text{l}/\text{mg}/\text{hr}$ )		
Dog No.	Before perfusion	After perfusion
67	2.20	1.15
69	2.52	2.01
71	2.35	1.94
Mean rate of decrease (%)	27.9	

**Table 22** Effect of perfusion, combined with storage at a low temperature, on tissue respiration of the free graft of sigmoid colon.

Perfusate : 5% low molecular weight dextran solution, added with heparin and xylocaine.

Dog No.	( $\mu$ l/mg/hr)		
	Before perfusion	After perfusion	After perfusion and storage
65	2.15	1.73	1.23
66	2.51	1.36	0.65
68	2.32	1.69	0.55
Mean rate of decrease (%)		31.5	65.0

**Table 23** Effect of perfusion, combined with storage at a low temperature, on tissue respiration of the free graft of sigmoid colon.

Perfusate : 5% low molecular weight dextran solution, added with heparin, xylocaine and chlorpromazine.

Dog No.	( $\mu$ l/mg/hr)		
	Before perfusion	After perfusion	After perfusion and storage
67	2.20	1.15	0.35
69	2.25	2.01	0.79
71	2.35	1.94	0.74
Mean rate of decrease (%)		27.6	74.0

### SUMMARY AND DISCUSSION

After an extensive resection of the esophagus including the cancer lesion, pedunculated segments of the digestive tract, such as stomach, jejunum, and colon, or complete free segment of the digestive tract are usually transplanted into the cervical, antethoracically subcutaneous, substernal or intrathoracic regions to perform reconstruction of the esophagus. Circulatory disturbance of the transplanted segment of the digestive tract resulting in sutural insufficiency at the site of anastomosis with the esophagus may ensue on account of the severance of the nutritional blood vessel, bilateral vagotomy<sup>1)41)</sup>, flexion or compression of the nutritional vessel and thrombus formation at the site of vascular reconstruction, due to the transplantation into an abnormal site<sup>1)16)</sup>. Moreover, even when the reconstruction of the esophagus was successfully carried out, postoperative change may occur in the secretion and motor function of these segments<sup>2)3)40)45)</sup> or postoperative reflux esophagitis may occur due to the loss of the cardiac sphincter mechanism at the esophagogastric junction<sup>8)15)48)</sup>.

According to the author, the biological nature or property of the tissue itself of each section of the digestive tract used for the reconstruction of the esophagus might play an important role in these problems. Using WARBURG manometer, the state of tissue respiration of the esophagus and various parts of the digestive tract used for the reconstruction of the esophagus was experimentally studied from various points of view.

At first, tissue respiration was determined in various parts of the digestive organ of a healthy dog: the esophagus, stomach, duodenum, jejunum, ileum, ascending colon, transverse colon, sigmoid colon and the liver. Moreover, the tissue respiration was determined in a part, which seemed to be healthy, i. e. the esophagus and stomach resected from a patient with cancer of the esophagus. The tissue respiration was the highest in the duodenum, followed by ileum, liver, jejunum, stomach, ascending colon, transverse colon, sigmoid colon, and finally the esophagus. In the time course of changes in tissue respiration, on the other hand, the speed of increase was slower in the esophagus than in the stomach. According to the report of CHISHIMA et al.<sup>6)</sup> the tissue respiration is high in the stomach where the pathological secretion is great in the presence of gastroduodenal ulcer and other parts of the digestive tract with intense secretory digestive and absorptive



function. The esophagus with only the role of conduit for food should have a low tissue respiration. These results agreed with the experimental results of IKEDA et al.<sup>14)</sup> on the tissue respiration of the mucous membranes of the upper digestive tract. The low tissue respiration of the esophagus might explain our clinical observation on the strong resistance of the esophageal tissue against hypoxia due to circulatory disturbance and the results of the experiment by MACMANUS on the extent to which one may interfere with the blood supply of the esophagus in dogs<sup>24)</sup>, along with the extremely low content of the autotissue protein splitting enzyme, cathepsin in the esophagus, as compared with other parts of the digestive tract, as demonstrated by MATSUO<sup>28)</sup> of our Department. From the standpoint of tissue respiration, on the other hand, resistance against ischemia due to circulatory disturbance may be the greatest in the colon, followed by the stomach, jejunum and ileum upon reconstruction of the esophagus using free or pedunculated segment of the digestive tract.

When the reconstruction of the esophagus was carried out using a segment of the gastrointestinal canal, bile reflux into the esophagus may be seen<sup>8)15)48)</sup> due to the loss of so-called cardiac sphincter mechanism at the esophagogastric junction. Examination of the influence of bile and bile acid on the tissue respiration of various parts of the digestive tract revealed a marked inhibition on these of the stomach, esophagus, sigmoid colon, jejunum and the liver<sup>44)</sup>, respectively. Concerning the etiology of the reflux esophagitis, TAKATSUKI<sup>43)</sup> in our Department demonstrated that the protein of the esophageal mucosa was more resistant to acid-pepsin and trypsin digestion than the protein in the mucosa of other parts of the digestive tract. MATSUO in our Department,<sup>28)</sup> on the other hand, demonstrated a slight activation of the trypsin activity by bile especially the bile acid, at the concentration in the intestinal content. Function of bile and bile acid to increase the infiltrating ability of hydrochloric acid into the esophageal membrane was also demonstrated by MATSUO. The results obtained by these workers as well as by the author might serve to elucidate the role of the bile and bile acid in the pathogenesis of reflux esophagitis. On the other hand, sigmoid colon and jejunum appear to be excellent transplants due to the smaller susceptibility to the inhibition of tissue respiration by bile and bile acid. This might be explained by the contact of these tissues, jejunum and liver, with a concentrated bile under the physiological condition so that their tissue respiration is less susceptible to bile.

Concerning the function of the gastric tube used for the reconstruction of the esophagus, KUDO<sup>23)</sup>, SHIMODA<sup>47)</sup> YAMAGUCHI<sup>48)</sup> etc. reported on the secretion and motility functions. Although these functions showed a pronounced decrease postoperatively, the recovery was usually seen after 1-6 months, especially upon antethoracic transplantation. Upon examination of tissue respiration in the antethoracically and intrathoracically transplanted gastric tubes in comparison with the gastric remnant in the abdominal cavity and the esophagus, the higher tissue respiration of the antethoracically transplanted gastric tube as compared with the intrathoracically transplanted one was demonstrated, showing an excellent agreement with the state of functional recovery in clinical cases<sup>23)31)40)</sup>.

Cytochrome C has recently been used for various vascular disturbances<sup>7)10)11)20)22)35)36)</sup>. The energy required for the maintenance of life is produced by the metabolism of sugar, protein, and fat ingested as food in relation to Krebs-cycle as well as by the combination of hydrogen thereby produced with the oxygen introduced through respiration to produce

water in a complicated metabolic process. The cytochrome system, which transports the electron produced through the activation of the above-mentioned hydrogen by the oxidation-reduction enzyme system such as dehydrogenase and yellow ferment to the oxygen, is indispensable in the process of tissue respiration. From the consideration on these points, the author attempted to apply the cytochrome C, a member of cytochrome system, nicotinic acid amide and activated vitamin B<sub>2</sub> or FAD, coenzymes of dehydrogenase and yellow ferment, to the reconstruction of the esophagus for the purpose of alleviating the influence of circulatory disturbance in the segment of the digestive tract for the reconstruction of the esophagus. At first, in the *in vitro* experiment, it was made clear that the use of cytochrome C alone or combination of active vitamin B<sub>2</sub> and nicotinic acid amide markedly elevated the tissue respiration of the esophagus and the stomach. The use of all three, cytochrome C, active vitamin B<sub>2</sub> and nicotinic acid amide gave a more distinct effect. In an *in vivo* experiment, a ROUX's pedunculated jejunal loop was prepared in an experimental dog and elevated into the subcutaneous tissue of the anterior chest. Upon measurement of the hemodynamic state with <sup>32</sup>P labeled red cells to assess the effect of these drugs, the use of cytochrome C alone and the combination of vitamin B<sub>2</sub> and nicotinic acid amide with cytochrome C elevated the efficiency of oxygen utilization especially markedly, making it possible to let the portion with marked circulatory disturbance survive. These results might indicate the possibility that the use of these drugs might reduce the frequency of the development of sutural insufficiency in esophageal reconstruction. This apparently opens a new field in the application of cytochrome C. Excellent results may be expected especially upon the combined use with the postoperative oxygen therapy after esophageal reconstruction whose validity we have already verified<sup>(5)(16)(17)(34)</sup>. The use of active vitamin B<sub>2</sub> and nicotinic acid amide together with cytochrome C is absolutely necessary as the results of our experiment indicated.

Although cytochrome C is excreted with a considerable rapidity from a healthy organism<sup>(3)(10)(35)(36)(37)(46)</sup>, it could exert its action passing through the protoplasmic membrane in a state of persistent hypoxia according to BÉRAUD<sup>(4)(7)</sup>. The effect is therefore expected on the segment of the digestive tract with circulatory disturbance. Although it is an interesting fact that the effect of cytochrome C was distinct in tissue with muscular layer, tissue specificity of the drug cannot be ignored.

In the complete free transplantation of the segment of digestive tract which has been conducted frequently in recent years due to the progress of the vascular surgery, the storage of the transplant outside of the body for a certain period provides an important problem in homo- or heterotransplantation between different donors and recipients as well as in division of operative procedures in autotransplantation to alleviate the operative insult. As the method of storage of complete free intestinal graft, HOSHINO et al.<sup>(13)</sup> recommended the storage by covering it with wet gauze instead of keeping it in the fluid. The optimum temperature was 4°C rather than -20°, 0° and 10°C. As the solution for storage, the use of dextran alone was slightly inferior, but RINGER and TYRODE solutions were equally good. The author adopted the method of storage at 4°C for 48 hours, covering it with wet gauze under a low temperature to follow up the changes in tissue respiration. While a marked decrease was seen in the gastric transplants, only a very mild

decrease was seen in the transplants from the sigmoid colon. This might give a valuable suggestion on the selection of the organ from which the transplants are to be taken. HOSHINO et al.<sup>13)</sup> also pointed out the gradual decrease in oxygen consumption as the days of storage increase. Ten days' storage at 4° C or 7 days at -20°C decreased the oxygen consumption immediately after resection to one-half the original level. This stage is the borderline of the successful transplantation according to him. Opinions have not reached an agreement concerning the need of the pretransplantation perfusion for the obtained free transplant. In our study on the effect of tissue respiration of sigmoid colon transplant perfused by the 5% low molecular dextran solution with the addition of heparin and xylocaine, a considerable decrease following perfusion was demonstrated. Although the perfusion of the transplant with low molecular weight dextran solution has the advantage of preventing the sludging which follows reopening of the blood stream, it also has the disadvantage of dissolving and washing out the lyoenzyme type of respiratory enzyme or low molecular weight coenzyme of respiratory enzyme. Although EYAL et al.<sup>9)</sup> reported on the prevention of the dissolution of soluble respiratory enzyme upon the addition of 10-12.5 mg of chlorpromazine in 200 cc of 5 % dextran, the effect was weak according to our experiments for confirmation. MATSUI in our Department demonstrated that the perfusion with low molecular weight dextran helped to keep the patency of the A-V anastomosis in the transplant following reopening of the blood stream. From these results, injection of heparin into the nutritional artery upon obtaining the transplant may suffice without perfusion when the blood stream is reopened a short time after the removal of the transplants. ODAKA<sup>33)</sup> and AOKI<sup>2)</sup> of Prof. NAKAYAMA's Department of Surgery, Chiba University also denied the need of perfusion and even pointed out its harmful effect when the transplants are kept at room temperature and transplanted within 4 hours. However, when the reconstruction of the blood stream once fails, perfusion with 100 cc of dextran G with the addition of heparin and 5 mg of isoxsuprine hydrochloride, a vasodilatator is recommended according to these authors.

#### SUMMARY

In order to study several points in the esophageal reconstruction using complete free or pedunculated segment of digestive tract such as stomach, jejunum, and colon from the standpoint of tissue respiration, experiments were carried out using WARBURG manometer and the following results were obtained :

1) The tissue respiration of various portions of the digestive tract, beginning with the esophagus, revealed the highest value in the duodenum, followed by the jejunum, ileum, stomach, ascending colon, transverse colon, sigmoid colon, and finally the esophagus.

2) Bile and bile acid exerted an inhibitory action on the tissue respiration of various parts of the digestive tract. The degree of inhibition was most pronounced in the stomach, followed by the esophagus, sigmoid colon, jejunum and the liver.

3) Cytochrome C, alone or in combination with active vitamin B<sub>2</sub> and nicotinic acid amide, activated the tissue respiration of various parts of the digestive tract, elevated the efficiency of oxygen utilization, alleviated the influence of the circulatory disturbance of the antethoracically elevated pedunculated jejunal loop and helped to prevent the sutural insufficiency at the site of anastomosis. The effect was more pronounced upon the use

of cytochrome C together with active vitamin B<sub>2</sub> and nicotinic acid amide than upon the use of cytochrome C alone.

4) The effect of the environment of transplantation upon the function of the gastric tube used for esophageal reconstruction was studied from the standpoint of tissue respiration. The tissue respiration of the antethoracically transplanted gastric tube was slightly greater than the intrathoracically transplanted one.

5) Storage at 4°C for 48 hours markedly decreased the tissue respiration of free transplant of the stomach, while the tissue respiration of the sigmoid colon transplant was only slightly decreased. Perfusion with 5% low molecular weight dextran with the addition of heparin and xylocaine markedly decreased the tissue respiration of the free transplant of sigmoid colon.

From these results, valuable data were obtained for the selection of the segment for the reconstruction of the esophagus from the digestive tract, the elucidation of the pathogenesis of reflux esophagitis, prevention of the sutural insufficiency at the site of anastomosis upon esophageal reconstruction through medication, and the management and storage of the segment of the digestive tract for the complete free transplantation.

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#### REFERENCES

- 1) Akakura, I. : On the difficulties encountered by operation for esophageal cancer. J.J.A.T.S., **8** : 6, 602-615, 1960.
- 2) Aoki, T. : Fundamental studies on the transplantation of the free intestinal graft, with special reference to the influence of medicines and perfusion on blood vessels. J.J.S.S., **66** : 13, 2030-2050, 1965.
- 3) Beinert, H. : Studies on the metabolism of administered cytochrome c by the aid of iron-labeled cytochrome. Science, **111** : 2887, 469-470, 1950.
- 4) Béraud, Th. & A. Vannotti : Variations du taux du cytochrome c dans les états d'inanition chez le lapin. Schweiz. med. Wschr., **85** : 8, 174-178, 1955.
- 5) Binet, L. & M. Bochet : Oxygénothérapie. deuxième édition, 1960, Masson & Cie, Paris.
- 6) Chishima, H. : Studies on tissue respiration of the gastric mucous membrane. Shinshu-Igaku-Zasshi, **8** : 8, 1625-1639, 1959.
- 7) Dutkiewicz, J. S. et al. : The effect of Cytochrome c on the oxygen consumption of tissues of normal and CO-poisoned animals. J. Physiol., **152** : 482-486, 1960.
- 8) Ellis, F.H.Jr. & R.T. Hood : Experimental esophagogastratomy ; Relation of type of resection to development of esophagitis. Surg. Gynec. & Obst., **98** : 4, 449-455, 1954.
- 9) Eyal, Z. et al. : Successful in vitro preservation of the small bowel, including maintenance of mucosal integrity with chlorpromazine, hypothermia, and hyperbaric oxygenation. Surgery, **57** : 2, 259-268, 1965.
- 10) Feinen, F.J. : Die Wirkung von Cytochrom c bei der Narkose und verschiedenen Sauerstoffmangelzuständen. Dtsch. med. Wschr., **80** : 4, 146-148, 1955.
- 11) Fukai, H. & S. Nakamura : Experience in the use of cytochrome c (cytochron) in the field of brain surgery. No-to-Shinkei, **15** : 6, 597-603, 1963.
- 12) Fujita, A. : Manometric technique and its application for the medical and biological researches. 1949. Iwanami-Shoten, Tokyo.
- 13) Hoshino, Y. et al. : Experimental studies on the transplantation of small intestine preserved at a low temperature. J.J.A.T.S., **13** : 7, 620-622, 1965.

- 14) Ikeda, K., S. Minami & S. Izaki : Studies on the tissue respiration of mucous membranes of the upper alimentary tract. *J.J.S.S.*, **65** : 8, 632-639, 1964.
- 15) Ishigami, K. et al. : Reflux esophagitis in the esophageal surgery, with special reference to its pathogenesis. *Nihon-Rinsho*, **16** : 8, 1233-1248, 1958.
- 16) Ishigami, K. et al. : Successful methods in the antethoracic esophagogastrostomy. *Geka-Chiryō*, **5** : 5, 511-523, 1961.
- 17) Ishigami, K. et al. : Operation for cancer of the mid-thoracic esophagus, with special reference to the postoperative oxygen therapy to succeed in the antethoracic esophagogastrostomy. *Shujutsu*, **16** : 4, 289-300, 1962.
- 18) Ishikawa, Y. et al. : Consideration of the complications after radical operation for cancer of the thoracic esophagus. *Shujutsu*, **14** : 12, 1028-1037, 1960.
- 19) Ito, F. : Experimental studies on incomplete sutures of gastroesophageal anastomosis undertaken below the skin in front of the thoracic wall and within the thoracic cavity. *Nagoya-Igaku*, **74** : 3, 557-569, 1957.
- 20) Kase, M. et al. : Cytochrome c. Its clinical application. *Chiryō*, **42** : 9, 1706-1711, 1960.
- 21) Katsura, S. & Y. Ishikawa : Jejunal transplantation to the resected portion of the esophageal cancer. *J. Japan. Practical Surgeon Soc.*, **18** : 3, 141-144, 1957.
- 22) Kobayashi, T. et al. : The use of cytochrome c in combating cardiovascular diseases. *Chiryō*, **44** : 1, 83-92, 1962.
- 23) Kudo, K. : Secretory function of the residual stomach after intrathoracic esophagogastrostomy. *Chiba-Igakukai-Zasshi*, **29** : 3, 291, 1953.
- 24) Macmanus, J. E., J. T. Dameron & J. R. Paine : The extent to which one may interfere with the blood supply of the esophagus and obtain healing on anastomosis. *Surgery*, **28** : 1, 11-23, 1950.
- 25) Majima, M. : Experimental studies on the healing mechanism at the suture portion of antethoracic esophagogastrostomy or-jejunosomy, with special reference to the pathogenesis of necrosis occurring in the antethoracically, subcutaneously transplanted gastrojejunal segments. *Arch. Jap. Chir.*, **28** : 5, 1766-1783, 1959.
- 26) Matsumoto, T. : Studies on esophageal reconstruction by means of the pedunculated gastric tube with additional micro-vascular anastomoses. *Arch. Jap. Chir.*, **34** : 5, 1118-1136, 1965.
- 27) Matsumura, A. : Studies on the measurement of the circulating blood cells by radioactive isotope P<sup>32</sup>. *Chiba-Igakukai-Zasshi*, **31** : 4, 455-462, 1955.
- 28) Matsuo, Y. : Experimental studies on the causes of reflux esophagitis, with especial emphasis on the significance of esophageal catheptase, bile and bacterial infection. *Arch. Jap. Chir.*, **28** : 6, 2002-2027, 1959.
- 29) Nakayama, K. : New technique for subtotal esophagectomy and some considerations in making a gastric tube. *Geka*, **14** : 12, 661-667, 1952.
- 30) Nakayama, K. : Surgical operations of the digestive organs. I, 1st edition, Kanehara Co. Ltd., 1954.
- 31) Nakayama, K. : Relation between the surgical operations of the digestive organs and the digestion or absorption. *J.J.S.S.*, **59** : 6, 972-989, 1958.
- 32) Noguchi, M. : Effects of temperature on the tissue respiration. II. Its influence on the tissue respiration of the viscera. *Jikei-Igakukai-Zasshi*, **75** : 5, 1037-1046, 1959.
- 33) Odaka, M. : Transplantation of the free autograft of the intestine for the cicatricial stenosis of the esophagus, with special reference to the operative method. *J.J.A.T.S.*, **12** : 3, 198-224, 1964.
- 34) Oyama, T. : Practice of the oxygen therapy. 1st edition, 1960, Nagai-Shoten, Osaka.
- 35) Proger, S. & D. Dekaneas : Some effects of injected Cytochrome c in myocardial and cerebral anoxia in man. *J. Pediat.*, **29** : 6, 729-736, 1946.
- 36) Proger, S. & D. Dekaneas : The use of Cytochrome c in combating tissue anoxia. *Science*, **104** : 2704, 389-390, 1946.
- 37) Rabinovitch, R., K. A. C. Elliott & D. McEachern : Cytochrome c : Intravenous administration in man. *J. Lab. & Clin. Med.*, **33** : 3, 294-296, 1948.
- 38) Sasakawa, Y. & T. Sekine : The use of Warburg's manometric technique, Method for enzymological studies. **1** : 509-605, 1955, Asakura-Shoten, Tokyo.
- 39) Seki, T. et al. : Warburg's manometer. *Kagaku-no-Ryoiki*, Suppl. 20, 1962, Nankodo, Tokyo-Kyoto.
- 40) Shimoda, T. : Studies on the functions of the stomach after the performance of antethoracic esophagogastrostomy. *Chiba-Igakukai-Zasshi*, **30** : 2, 177-182, 1954.
- 41) Sugiura, N. : Impaired hood circulation in the gastric tube utilized for esophageal reconstruction as a sequel to vagotomy, with special reference to sympathectomy as a countermeasure. *Arch. Jap. Chir.*, **35** : 1, 1-23,

- 1966.
- 42) Suzuki, H. : Experimental studies on antethoracic esophageal reconstruction by the utilization of the jejunal loop transplanted into the pectoral muscle, with especial reference to blood circulation in the loop. Arch. Jap. Chir., **29** : 1, 93-124, 1960.
- 43) Takatsuki, H. : Experimental studies on the pathogenesis of reflux esophagitis, with special reference to the resistance of esophageal mucosa to peptic or tryptic digestion. Arch. Jap. Chir., **28** : 6, 2087-2107, 1959.
- 44) Takeuchi, Y., K. Nakamura & K. Kato : On the effects of the some chemical agents on the tissue respiration of gastric and duodenal mucous membrane. J.J.S.S., **59** : 6, 862-863, 1958.
- 45) Tsukuda, M. : Experimental studies on the antethoracic esophageal reconstruction, with particular reference to the development of new vessels for the antethoracically, subcutaneously transplanted gastrojejunal segments. Arch. Jap. Chir., **28** : 6, 2222-2245, 1959.
- 46) Werth, G. : Betrachtungen zur therapeutischen Verwendung des Cytochroms c. Dtsch. med. Wschr., **81** : 7, 249-250, 1956.
- 47) Yamamura, Y. & M. Kusunose : Succinic dehydrogenase. Method for enzymological studies. **2** : 496-506, 1956, Asakura-Shoten, Tokyo.
- 48) Yamaguchi, I. : Postoperative anorexia as one of the complaints after radical operation for esophageal and cardiac carcinoma and postoperative reflux esophagitis. Part 2. Reflux esophagitis. J.J.A.T.S., **5** : 13, 1176-1186, 1957.
- 49) Yoshikawa, H. et al. : Warburg's manometer. Kagaku-no-Ryoiki, Suppl. 13, 1960, Nankodo, Tokyo-Kyoto.

## 和文抄録

### 組織呼吸の面からみた食道再建術に関する2, 3の問題

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食道切除につづいて、胃、小腸あるいは結腸などの消化管分節の有茎移植片または完全遊離移植片を用いて食道再建術を行なう際、直接問題となる点や術後の逆流性食道炎および移植胃腸管の遠隔時機能などにおいては、食道および食道再建に使用される消化管各部位のもつ組織自体の生物学的な本性あるいは属性が本質的な役割を演じていることは想像に難くない。このような点を考慮して、著者は食道を初めとする消化管各部位の組織呼吸をWarburg検圧法を用いて色々の観点から測定し、以下の成績を得た。

1) 食道を初めとする消化管各部位の組織呼吸量を実験犬および食道癌患者から採取された材料について測定し、十二指腸、回腸、肝臓、空腸、胃、上行結腸、横行結腸、S字状結腸、食道の順に低値を示すことを明らかにした。食道組織が血行障害に伴なう低酸素症に対してかなり強い抵抗性をもっていることは一部このことに基づくものと思われる。またこの成績は

食道再建術の際、結腸、胃、空腸、回腸の順に血行障害または乏血によく耐えることを示している。

2) 食道再建術後の合併症である逆流性食道炎や吻合部縫合不全の原因として胆汁の関与が考えられるので、消化管各部位の組織呼吸に対する胆汁および胆汁酸塩 Sodium taurocholate の影響を検討し、胃、食道、S字状結腸、空腸、肝臓の順に著明な抑制がみられることを明らかにした。

3) 食道再建用Kirschner・中山式胃管の遠隔時機能に対する移植環境の影響を組織呼吸の面から検討し、移植3ヵ月後には胸郭前および胸腔内移植胃管の組織呼吸量は手術操作を加えていない胃、腹腔内残胃および食道のそれらと比較するといずれも低下しており、しかも胸腔内移植胃管の組織呼吸量は胸郭前移植のそれより低値を示すことを知った。

4) チトクロームCおよび脱水素酵素の補酵素であるニコチン酸アミドや黄色酵素の補酵素である活性

型ビタミンB<sub>2</sub>, すなわち FAD の食道および胃の組織呼吸におよぼす影響を In vitro で検討し, これらの薬剤は組織呼吸の効率を著明に高め, さらにチトクロームC, 活性型ビタミンB<sub>2</sub>およびニコチン酸アミドの3者を併用した場合にはより著明な効果が得られることを明らかにした。次に In vivo の実験として, 実験犬において Roux 式有茎空腸管を作製して胸郭前皮下に挙上し, <sup>32</sup>P 標識赤血球を使用してその血行動態を測定した後, これらの薬剤投与の効果を検討したところ, チトクロームCを単独に投与した場合には勿論のこと, これに活性型ビタミンB<sub>2</sub>とニコチン酸アミドを併用した場合にはとくに酸素の利用効率を著明に高め, 血行障害の著明な部位をも生存せしめ, ひいては食道再建術の際の縫合不全の発生の予防に役立つことを明らかにした。

5) 消化管分節の完全遊離移植の際に問題となる移植片の処理および体外保存の方法を組織呼吸の面から検討した。移植片を生理的食塩水でしめたガーゼでおおい, 4°Cに48時間保存すると, 組織呼吸量は胃移

植片では48.3%低下したが, S字状結腸移植片では4.9%の低下を示すにすぎず, 保存遊離移植片としては後者の方がすぐれていると考えられる。一方5%低分子量デキストランにヘパリン32mgおよび4%キシロカイン5ccを加えた灌流液で灌流を行なうと, S字状結腸移植片の組織呼吸量は灌流前に比べて31.4%も減少することを明らかにした。これは灌流によつて溶解型呼吸酵素や低分子の呼吸酵素補酵素が溶出することを示し, 移植片採取後短時間内に血行が再建される際には灌流はむしろ有害であるという臨床経験のよつて来るところを一部説明するものである。以上の灌流液中にクロールプロマジン12.5mgを添加すると, 組織呼吸量の低下は27.9%とやや軽度となつた。

以上の諸成績から, 食道再建術の際に問題となる食道再建用消化管分節の採取部位の撰択, 逆流性食道炎の成因の解明, 食道再建用移植胃管の遠隔時機能の解明, 薬剤投与による食道再建術の際の吻合部縫合不全の防止, 完全遊離移植用消化管分節の処理または体外保存の方法などについて貴重な参考資料を得た。