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Histochemical Studies on the Gland Cells.

I. Secretory Activity and Intracellular Amino Acid of the Gastric Chief Cells.

腺細胞の組織化学的研究.

I. 胃腺主細胞の分泌機能と細胞内アミノ酸について.

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Previously, FUJIE employed the chemical colour reactions for protein and amino acids on the sections of dogs' gastric mucosa using the histochemical methods. He found that the reactions for the imidazol group were always positive in the sub-nuclear portion of the gastric surface epithelial cells, where *productin* vacuoles (FUJIE) were seen. The findings will throw light on the research on the true nature of FUJIE's gastric hormone *productin*.

Here, the relation between the component of the gastric hormone *productin* and that of the secretory granules in the gastric chief cells as produced in the cell affected by the *productin*, presents an interesting study, for which reason the authors have attempted this study here.

I. Materials and Methods.

The experimental animals are adult and male albino rats which were accustomed to the regular feeding of Mc COLLUM's artificial diet.

Materials were taken from two experimental groups of rats as follows:

1. The artificial diet was given to rats which were without food for 24 hrs. The rats were killed at the periods of a) before administration of the diet, and b) $\frac{1}{2}$ and 1 hr. after administration of the diet.

2. The unfed rats (24 hrs. after the last diet) were injected subcutaneously with 3 mg of Histamine·HCl. They were killed at the periods of $\frac{1}{3}$, $\frac{2}{3}$ and 1 hr. after the injection.

To take materials, under deep ether narcosis the abdomen was opened and the stomach was taken out. Half of it was placed at once in 10% formalin liquid and the other half was in KOLSTER's liquid. Paraffin sections of 4—7 μ were made. The sections obtained from the stomach fixed by KOLSTER's liquid were stained by HEIDENHAIN's iron haematoxylin. The sections of the stomach fixed by formalin liquid were used for the chemical colour reactions described below.

II. Observations and Discussion.

The gastric chief (peptic) cells were observed. The cytological observations were manifested using the sections stained by HEIDENHAIN's iron haematoxylin. The quantity of secretory granules or vacuoles in the cell was marked +++ when the upper half of the cell body was full of the granules or vacuoles, and it was marked — when no granules or vacuoles were seen. Between +++ and —, three intervening grades of ++, +, ± were used. The quantity of the secretory substance, the form of plastosomes in the cells and the aspect of the acinus lumen have been tabulated, so that the secretory activity of the cells might be readily comprehended.

Table 1. The cytological observations on the gastric chief cells.

Exp.	Hrs.	Total result			Representative result		Acinus lumen
		Quantity of the intra-cellular secretory substance		Form of plastosomes			
		Granules	Vacuoles		Granules	Vacuoles	
Unfed (Control)		— ~ + +	± ~ +	long rod, short rod, rosary form	— ~ +	± ~ +	less wide, narrow
Administration of artificial food	$\frac{1}{2}$	± ~ + +	± ~ + (+)	short rod, short rosary form, guitar form, long rod	+ ~ + +	± ~ +	less wide, narrow, wide
	1	± ~ + + (+)	+ ~ + +	short rod, guitar form, rosary form	+ ~ + + (+)	+ ~ + +	less wide, narrow, wide
Subcutaneous injection of histamine·HCl	$\frac{1}{3}$	+ ~ + +	± ~ +	short rod, guitar form, short rosary form	+ ~ + +	± ~ +	narrow, less wide
	$\frac{2}{3}$	+ ~ + + (+)	— ~ +	short rosary form, guitar form, short rod	+ +	±	narrow, less wide
	1	+ ~ + + +	— ~ +	guitar form, short rosary form, short rod, rosary form	+ + ~ + + (+)	— ~ +	narrow, less wide

It is to be noted from these observations that the production of secretory granules is initiated in the cell after administration of food, then the granules are vacuolized and discharged slowly from the cell, and that the production of secretory granules is more active when there has been an injection of histamine, than where there has been the administration of diet.

The results of chemical colour reactions employed on the paraffin sections obtained from the stomach fixed by formalin liquid are as follows (Table 2).

The reactions for phenol group (tyrosine) and imidazol group (histidine) are always positive, and those for indol group (tryptophan), SS and SH group are negative. In the positive reactions, intensive results are obtained from the MILLON reaction and the tetrazonium reaction. The authors, therefore, will describe in detail on these reactions.

Table 2. The chemical colour reactions on the gastric chief cells and the results obtained.

Reaction (r.)	The objective substance of the positive result	The authors' result
The biuret r.	Polypeptide, protein	$\pm ?$
The xanthoproteic r.	Tyrosine, phenylalanine, tryptophan	$\pm \sim + (?)$
The ninhydrin r. (Serra-Queiroz-Lopes)	α amino acid	$\pm ?$
The PAULY diazo-r.	Tyrosine, histidine	+
The HANKE-KOESSLER r.	Histidine	+
The MILLON r. (BENSLEY-GERSH)	Tyrosine	++ , or -
The COLE aldehyde r.	Tryptophan	-
The ROMIEU r.	Tryptophan	-
The tetrazonium r.	Tyrosine, histidine, tryptophan	+++
The tetrazonium r. after 2.4-dinitrofluorobenzen treatment	Histidine, tryptophan	$++ \sim +++$
The tetrazonium r. after performic acid treatment	Tyrosine, Histidine	+++
The tetrazonium r. after benzoyl chloride treatment		-
The PEARSE r.	SH or SS group	-

a) *The MILLON reaction.*

The modification after BENSLEY and GERSH were employed.

The upper half of the chief cells of the unfed rats is coloured diffusively orange $\sim$ rose red (Fig. 1). This colour is the positive result. Secretory vacuoles are colourless. *Lamina muscularis mucosae* and *tunica muscularis* react positively on the reaction and the erythrocytes are negative.

At the periods of $\frac{1}{2}$, 1 hr. after administration of food, when secretory granules increase in the cells (Table 1), for the most part cells are almost without reaction (Fig. 2), but *lamina muscularis mucosae* and *tunica muscularis* continue to react in the same way as in the unfed rats. However, the chief cells are coloured positively at the periods of $\frac{1}{3}$, $\frac{2}{3}$, 1 hr. after the injection of histamine, though there is a marked increase in the production of secretory granules in the cells at these periods (Table 1). The secretory granules produced in the cells are indiscernible in the case of this reaction. Secretory vacuoles are colourless.

b) *The tetrazonium reaction.*

The upper half of the chief cells of the unfed rats reacts positively on the reaction. At the periods of $\frac{1}{2}$, 1 hr. after administration of food, secretory granules in the cells react positively. The positive zone, therefore, seems to be granular rather than diffused. Secretory vacuoles are colourless.

In the case of histamine injection, not only the secretory granules but also the

intergranular cytoplasm is positively coloured. That is, the strongly coloured granular part can be seen in the positive zone (Fig. 3 and 4).

Comparing the results obtained here with those obtained from the MILLON reaction, we may say that the diffused positive colour may have resulted from phenol group.

In all these cases, *lamina muscularis mucosae*, *tunica muscularis* and erythrocytes react intensively on the reaction.

c) *The tetrazonium reaction after 2, 4-dinitrofluorobenzen treatment.*

The phenol ring of tyrosine can be blocked by the use of 2,4-dinitrofluorobenzen (DNFB) and the substance formed by the reaction is colourless by the tetrazonium reaction. Two cases of the incubation 5 hrs. and 24 hrs. in DNFB were tested, but no difference can be seen between them.

The upper half of the chief cells of unfed rats react mildly. When a few secretory granules scatter there, they are distinguishable. Secretory vacuoles are colourless as they were in the other previous reactions. At the periods of $\frac{1}{2}$, 1 hr. after administration of food and the periods of $\frac{1}{3}$, $\frac{2}{3}$, 1 hr. after the injection of histamine, the reaction obtains similar results; that is, only the secretory granules are strongly coloured in the chief cells. This is shown in Fig. 5. *Lamina muscularis mucosae*, *tunica muscularis* and erythrocytes continue to react.

d) *The tetrazonium reaction after performic acid treatment.*

After treatment with performic acid, the indol ring of tryptophan fails to react by the tetrazonium reaction. The results of the reaction obtained from each experimental case are very similar to those of the tetrazonium reaction alone (b).

e) *The tetrazonium reaction after benzoyl chloride treatment.*

By the use of benzoyl chloride, the subsequent reaction of tyrosine, tryptophan and histidine by the tetrazonium reaction should be prevented. Two cases of the incubation 16 hrs. and 24 hrs. in benzoyl chloride were tested.

In both cases, the chief cells, *lamina muscularis mucosae*, *tunica muscularis* and erythrocytes do not react at all on the tetrazonium reaction except for the nucleus of the cell (Fig. 6). The negative results have no relation with the experimental conditions.

Positively coloured granules can be seen in the mast cells and the apical portion of the surface epithelial cells. The former may be the granules of histamine (LAGUNOFF et al.) and the latter may be mucigen granules (PEARSE).

The results of these reactions are summarized in Table 3. Secretory granules in the gastric chief cells contain the imidazol-substance and the portion in which the granules are produced contains the phenol-substance. In the case when food was given, the phenol-substance decreases and disappears as the secretory granules are produced, but in the case when the production of the secretory granules are promoted by the injection of histamine, no decrease and no disappearance of the phenol substance can be seen. Secretory vacuoles are colourless by the reactions as regards the phenol-group and the imidazol-group.

According to these findings, the authors will discuss as follows. It is already known that the secretory granules in the gastric chief cells of rats are the secretory

substance produced in the cell and they are stained by HEIDENHAIN's iron haematoxylin, whilst on the other hand, the secretory vacuoles are the secretory substance which is discharging from the cell and are in fact nothing else but the secretory granules which have been lost their stainability. Because of the affinity of secretory granules to iron haematoxylin, it is obvious that the composition of the secretory granules is different from that of the secretory vacuoles. The authors proved here the difference according to the chemical colour reactions and went a step further showing their qualitative dissimilarities.

Table 3. The detail of the results obtained from the colour reactions.

Reac.	Exp.	Hrs.	Upper half of the cell body	Secretory granules	Secretory vacuoles
MILLON r.	Administra- tion of food	Unfed	+ ~ ++	?	—
		$\frac{1}{2}$	— ~ $\pm$	?	—
		1	— ~ $\pm$	?	—
	Injection of histamine	$\frac{1}{3}$	+ ~ ++	?	—
		$\frac{2}{3}$	++	?	—
		1	+ (+) ~ ++	?	—
Tetrazonium r.	Administra- tion of food	Unfed	++	? (+)	— ~ $\pm$
		$\frac{1}{2}$		++	—
		1		++	—
	Injection of histamine	$\frac{1}{3}$	+	++	—
		$\frac{2}{3}$	+	++	—
		1	+	++	—
2·4·D. N. F. B. and tetrazonium r.	Administra- tion of food	Unfed	+ ~ ++	(+ ~ ++)	—
		$\frac{1}{2}$		++	—
		1		++	—
	Injection of histamine	$\frac{1}{3}$		++	—
		$\frac{2}{3}$		++	—
		1		++	—
Performic acid and tetraz. r.	Administra- tion of food	Unfed	++	? (+)	—
		$\frac{1}{2}$		++	—
		1		++	—
Benz. chloride and tetraz. r.	Administra- tion of food	Unfed	—	—	—
		$\frac{1}{2}$	—	—	—
		1	—	—	—

The fact that the phenol-substance (tyrosine) which is seen in the upper half of the chief cells of unfed rats disappears as the secretory granules are produced in the cell after administration of food, seems to suggest a consumption of phenol substance in order to produce the secretory granules (imidazol substance) in the same region. However, it is unthinkable that the phenol-ring of tyrosine is changed into the imidazol-ring of histidine. The consumption of phenol substance, therefore, may be as a result of the participation of the substance during the production of the imidazol-substance (secretory granules).

In the case of the subcutaneous injection of histamine, it is noticed that the production of secretory granules (the imidazol-substance) is remarkably active in the chief cells and that the phenol-substance does not decrease or disappear. This may be due to the fact that the imidazol-substance (secretory granules) is produced without the participation of the phenol-substance because the injected histamine is itself an imidazol-substance, or that the productive activity of the secretory granules is so vigorous that the supply of the phenol-substance to the cells is increased.

III. Summary.

On the paraffin sections of the rats' stomach fixed by formalin liquid, the chemical colour reactions for protein and amino acids were employed by using the histochemical methods, and the gastric chief cells were observed.

The reactions for phenol-group and imidazol-group (PAULY diazo-reaction, HANKE-KOESSLER reaction, MILLON reaction, tetrazonium reaction and the reaction followed the 'blocking' reactions) are definitely positive, and the reactions for indol-group (COLE aldehyde reaction, ROMIEU reaction) and SH or SS group (PEARSE performic acid-SHIFF reaction) are negative.

Using the positive reactions, the chief cells were observed according to the phases of their secretory activity. The findings are summarized as follows.

Phenol-group (tyrosine) is proved to exist in the upper half region of the chief cells of unfed rats. The phenol-substance decreases and disappears as the secretory granules are produced in the same region in the cells after administration of food. Imidazol-group (histidine) is proved to exist in the secretory granules produced in the cells. The secretory vacuoles which are the secretory granules which have undergone a change are colourless by the reaction for both phenol-group and imidazol-group.

The subcutaneous injection of histamine brings about the vigorous production of secretory granules in the chief cells of unfed rats. The activity is marked during 1 hr. after the injection. At this period, no decrease and no disappearance of the phenol-substance in the upper half of the chief cells can be seen, and the imidazol-substance (secretory granules) is produced in the region where the phenol substance still exists.

内 容 自 抄.

フォルマリン固定, パラフィン切片のラッテ胃に対して蛋白質, アミノ酸に係る呈色反応を組織化学的方法で試み, 胃腺主細胞を観察した, フェノール核,

イミダゾール核に関係ある反応 (Pauly のジアゾ反応, Hanke-Koessler 反応, Millon 反応, テトラゾニウム反応及び封鎖処置後のテトラゾニウム反応) は陽性, インドール核に関係する反応 (Cole のアルデヒド反応, Romieu 反応) 及び SS, SH 基に関する反応 (Pearse による過蟻酸-Schiff 法) は陰性の結果を示す.

陽性結果を示す反応を用い, 胃腺主細胞分泌機能の活動時期別に観察したところ, 以下の如き所見を得た.

1. 空腹時, 即ち分泌機能休止期に於いては胞体上半にフェノール核物質 (クロジン) が証明される. 給食して胞体上半部に分泌顆粒が生成されると, フェノール核物質は証明されなくなり, 生成された分泌顆粒にイミダゾール核物質 (ヒスチジン) が証明される. 分泌空胞はいずれの反応にも呈色しない.

2. 空腹時に塩酸ヒスタミンを皮下注射すると, 主細胞には旺盛な分泌顆粒生成が見られるが, この時胞体上半部のフェノール核物質の消失がなくフェノール核物質の証明される部位にイミダゾール核物質たる分泌顆粒が生成され, 両物質が同時に共存することが証明される.

References.

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Explanation of Plate.

Fig. 1. The positive result of the MILLON reaction. The gastric mucosa of unfed rats. Upper half region of the gastric chief cells are coloured orange ~ rose red.

Fig. 2. The negative result of the MILLON reaction on the gastric chief cells. 1 hr. after administration of food. *Lamina muscularis mucosae* is positive, but it is obscure in the figure.

Fig. 3 and 4. The tetrazonium reaction on the chief cells after the injection of histamine. Secretory granules and the intergranular cytoplasm react on the reaction.

Fig. 5. The tetrazonium reaction after 2,4-dinitrofluorobenzen treatment. The gastric chief cells after the injection of histamine. Only the secretory granules in the cell react on the tetrazonium reaction.

Fig. 6. The tetrazonium reaction after benzoyl chloride treatment. The gastric chief cells and the muscle cells do not react at all except for the nuclei of the cells. 16 hrs. incubation in benzoyl chloride.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6