Histochemical Studies on Catecholamines in Paraganglia and Paraganglioma

by

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Received for Publication July 7, 1965

INTRDUCTION

It is generally accepted that chromaffin cells of the human foetus or young child are to be found both inside the adrenal gland and in the retroperitoneal tissues.

The extra-adrenal collections of chromaffin cells were nominated by A. KOHN (1903) [35)36)] as paraganglia.

Both intra-adrenal and extra-adrenal chromaffin cells are developed from the sympathogonia. The sympathogonia differentiates to the adrenal medulla and at the same time, differentiates to the paraganglia along the aorta and the sympathetic trunk.

In man, the paraganglia are commonly known as the "organs of ZUCKERKANDL"[58)59)] in the foetus and newborn infant. These organs were investigated extensively by IVANOFF (1925)[27)] and IWANOW (1930)[28)29)] and they concluded that the organs were degenerated and disintegrated in the earlier childhood.

On the other hand, the existence of the paraganglia in adult was reported by COUPLAND (1954)[11)].

NAKATA[41)] in our department, too, observed the paraganglia histochemically in the retroperitoneal tissues which were obtained operatively from the patients of peripheral vascular disease of the extremities on 1962. Since then, the paraganglia have been observed in many cases (Fig. 13).

Accordingly, whether the paraganglia may be some other source of catecholamines than the adrenal medulla and the adrenergic fibers, and whether they may be the tissues of origin in which the extra-adrenal pheochromocytomas[6)21)49)54)] originate, become the subjects of discussion.

The catecholamines in the paraganglia and the paraganglioma have been studied histochemically in this report.

MATERIALS AND METHODS

The experiment were performed on rabbits and dogs. The paraganglioma was obtained operatively.

As histochemical methods, HILLARP and HÖKFELT's technique[22)23)24)] was employed to demonstrate noradrenaline selectively and the chromaffin reaction[18)22)23)24)39)43)] was employed to demonstrate both adrenaline and noradrenaline.

A) HILLARP and HÖKFELT's technique[22)23)24)]

1) Fresh tissues were put into 10% potassium iodate solution for 48 hours,

2) fixed in 10% neutral formalin solution for 24 hours,
3) washed in running water for 24 hours,
4) dehydrated,
5) enclosed in paraffin, sliced,
6) stained with hematoxylin,
7) dehydrated and mounted.

B) The chromaffin reaction[18)22)23)24)39)43)]
1) Fresh tissues were put into 5% potassium bichromate (9 vol.) and 5 % potassium chromate (1 vol.) solution for 48 hours,
2) fixed in 10% neutral formalin solution for 24 hours,
3) washed in running water for 24 hours,
4) dehydrated,
5) enclosed in paraffin, sliced,
6) stained with hematoxylin,
7) dehydrated and mounted.

By HILLARP and HÖKFELT's technique, noradrenaline is stained selectively in brown yellow. On the other hand, by the chromaffin reaction, adrenaline and noradrenaline are stained in dark brown yellow.

(1) Dogs were administered Aldomet (L-α-Methyldopa)[51)] 500mg orally daily for 20 days. Dogs were laparotomized under intravenous anesthesia (Nembutal 25mg/kg) and the retroperitoneal tissures were removed including the aorta and bilateral adrenal glands from the origin of celiac artery to the bifurcation of iliac artery. Immediately after removing the tissues, they were stained by the methods described before.

(2) Rabbits were injected reserpine[3)8)9)10)14)15)] (5mg/kg) intravenously. 24 hours after injection, rabbits were laparotomized under intravenous anesthesia (Nembutal 25mg/kg) and the retroperitoneal tissues were removed and stained.

(3) Rabbits were laparotomized under intravenous anesthesia (Nembutal 20 mg/kg) and the left paraganglion was transplanted in the left adrenal medulla. Three weeks later, bilateral adrenal glands were removed and stained.

(4) Rabbits were laparotomized under intravenous anesthesia (Nembutal 20 mg/kg) and unilateral adrenal cortex was excised. Three weeks later, bilateral adrenal glands were removed and stained.

(5) The extra-adrenal pheochromocytoma (paraganglioma)[6)21)54)49)] obtained operatively was stained (Fig. 2).

RESULTS

(1) Although the paraganglia had been considered to be the storage sites of catecholamines, their secretory activity had not been clarified.

In this report, to prove the secretory activity of the paraganglia, dogs were administered Aldomet (L α-Methyldopa)[51)] 500mg orally. 4 dogs were used in this experiment. But the histochemical differences were not observed between Altomet-treated and control groups in both paraganglia and adrenal medulla.

(2) To prove the secretory activity of the paraganglia more surely, rabbits were

injected reserpine[3)8)9)10)14)15)] (5mg/kg) intravenously. 12 rabbits were used in this experiment. In all cases, the pronounced loss of catecholamines in the paraganglia was observed (Fig. 3, Fig. 4).

The catecholamines were depleted from the adrenal medulla, either (Fig. 5, Fig. 6).

(3) The paraganglia contain noradrenaline chiefly[41)] while the adrenal medulla contains adrenaline chiefly. It is generally believed that noradrenaline is the immediate precursor of adrenaline[5)32)]. Accordingly, whether the adrenal cortex may act on the methylation of noradrenaline to adrenaline is subject to debate. This problem has been discussed by many investigators[7)47)48)56)].

8 rabbits were used in this experiment. The left paraganglion was transplanted in the left adrenal medulla. If the adrenal cortex will act on methylation, adrenaline will be expected to increase in the paraganglion transplanted in the adrenal medulla. But the satisfactory result was not obtained.

(4) To obtain a good result, the unilateral adrenal cortex excised conversely. 18 rabbits were used in this experiment. If the adrenal cortex will act on methylation, noradrenaline will be expected to increase in the adrenal medulla. But, by the potassium iodate method, no increase of noradrenaline was observed in the adrenal medulla compared with the normal one (Fig. 7, Fig. 8). What is more, the total amount of catecholamines was almost same in both cortex excised and normal adrenal medulla by the chromaffin reaction (Fig. 9, Fig. 10).

In conclusion, the action of adrenal cortex on methylation was not observed in this experiment. The methylation of noradrenaline is considered to occur only in the adrenal medulla.

(5) The extra-adrenal pheochromocytoma (paraganglioma)[6)21)49)54)] obtained operatively was examined histochemically (Fig. 12). The extra-adrenal pheochromocytoma in the clinical sense and the paraganglioma were discussed later.

DISCUSSION

The extra-adrenal collections of chromaffin cells were nominated by A. KOHN (1903) [35)36)] as paraganglia. The paraganglia had been observed in rabbits, cat and dog from old times[53)] and had been recognized to exist till the end of their life.

In man, ZUCKERKANDL (1901)[58)] observed the paraganglia in the vicinity of the origin of inferior mesenteric artery in the foetus which are commonly known as the "organs of ZUCKERKANDL"[58)59)]. The fact that the extract of these organs has the adrenaline-like effect was proved by BIEDLE and WIESEL (1902)[4)]. But the "organs of ZUCKERKANDL"[58)59)] were considered to be degenerated and disintegrated in the earlier childhood and considered not to exist in adult. From this fact, the paraganglia became not to be paid attention.

On the other hand, recently the paraganglia were observed in the vicinity of the origins of celiac and inferior mesenteric arteries by COUPLAND (1954)[11)]. NAKATA[41)] in our department, too, observed the paraganglia histochemically in the retroperitoneal tissues which were obtained operatively from the patients of peripheral vascular disease of the extremities on 1962. Since then, the paraganglia have been observed in many cases (Fig. 13). He also observed the paraganglia in rabbit, cat and dog.

NAKATA[41] proved histochemically that the paraganglia contain noradrenaline chiefly. Accordingly, whether the paraganglia may be some other source of catecholamines than the adrenal medulla and the adrenergic fibers, and whether they may be the tissues of origin in which the extra-adrenal pheochromocytomas[6)21)49)54] originate, become the subjects of discussion.

The paraganglia, in their large content of catecholamines, in their endocrine-like structure and in having preganglionic innervation[25], had been considered to be the powerful storage and secretory sites of catecholamines. But the secretory activity of paraganglia had not been clarified. NAKATA[41] observed that the catecholamines in the adrenal medulla was depleted by insulin-induced hypoglycemia while the catecholamines in the paraganglia did not be depleted. From this fact, he supposed that both paraganglia and adrenal medulla have the same chromaffinity but their secretory activities are different and independent.

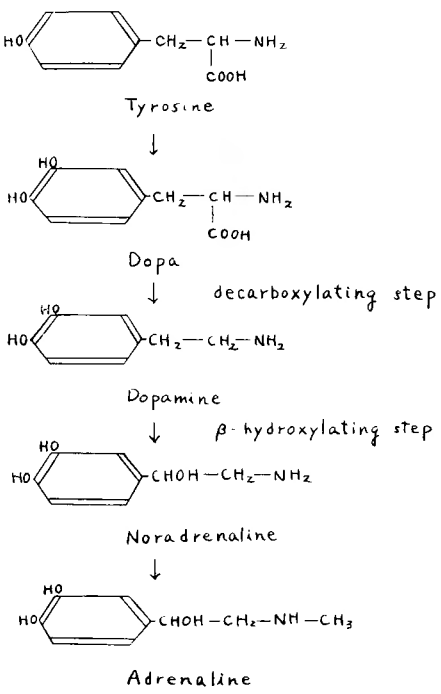

Fig. 1. Main pathway of formation of catecholamines.

The biosynthesis of catecholamines[5)32] is considered to be done as showed in Fig. 1.

Noradrenaline is not only the immediate precursor of adrenaline but has the role of hormone. As previously described, the loss of catecholamines in the paraganglia was not demonstrated by insulin-induced hypoglycemia[41].

With a view to demonstrating the loss of catecholamines in the paraganglia, Aldomet (L-α-Methyldopa)[51] was used. Aldomet is considered to interfere with the decarboxylating step in the amine synthesis. But the histochemical differences were not observed between Aldomet-treated and control groups in both paraganglia and adrenal medulla. Oral administration may be unstable in dogs.

A large number of studies have been published demonstrating a pronounced loss of adrenaline and noradrenaline from adrenal medulla after administration of reserpine[3)8)9)10)14)16]. Reserpine was used to prove the secretory activity of paraganglia. Not only the catecholamines in adrenal medulla but in paraganglia were depleted by reserpine.

The mode of action and the pharmacologic problems of reserpine on the adrenal medulla and paraganglia must be though over here. Reserpine was used intravenously at the rate of 5mg/kg. The paraganglia and the adrenal glands were stained 24 hours after administration of reserpine. These numerical values were adopted in the light of the experimental results obtained by many investigators. CALLINGHAM and MANN[8] used reserpine 1mg/kg subcutanously for 4 days and ERÄNKÖ and HOPSU[14] used 4mg/kg sub-

cutanously for 4 days. BERTLER et al[3]. injected single dose of reserpine 5mg/kg intravenously.

The recovery of medullary hormones after depletion induced by reserpine has been much less studied[8)14)] compared with the studies demonstrating a pronounced loss of catecholamines from adrenal medulla. By the injections of various doses of reserpine, the recovery periods of catecholamines were different. But 24 hours after administration of resepine, the most pronounced loss of catecholamines from adrenal medulla could be obtained.

Then, the mode of action of reserpine on the catecholamines was studied by BERTLER et al[3]. (1961). According to their studies, when L-dopa (100mg/kg) was given intravenously to reserpinized (5mg/kg) rabbits, appreciable amounts of dopamine and noradrenaline formed in the adrenal medulla. The results support the view that reserpine does not at least not to a great extent interfere directly with the decarboxylating and β hydroxylating steps in the amine synthesis.

At the same time, the action of reserpine to the secretory nerve through the central nervous system must be taken into consideration.

The two processes: storage and release are presumably balanced in the normal cell. Thus if the storage process is blocked, the result will be a depletion. The secretory activity of paraganglia was proved by these considerations.

The adrenal cortex and its relation to the methylation of noradrenaline have been discussed by many investigators[7)47)48)56)]. Embryonic tissue in the adrenal glands of man, cat, rabbit and dog contains a high proportion of noradrenaline and a very small amount of adrenaline[48)]. Large amounts of noradrenaline have also been found in the "organs of ZUCKERKANDL" of children aged less than 70 days[56)] ; this abdominal chromaffin tissue lacks connection with the adrenal cortical cells. In adult animals, however, methylation of noradrenaline is almost complete in the adrenal glands when the cortex is large relative to the medulla (e. q. rabbit and guineapig), but when the medulla is relatively large (e. q. whale and foul) methylation of noradrenaline occurs to a very small degree.

It was suggested[48)] therefore that the cortical size to medullary size may be related to the proportion of noradrenaline present in the adrenal gland.

To investigate this problem histochemically, to begin with, the paraganglion was transplanted in the adrenal medulla. If the adrenal cortex will act on the methylation, adrenaline will be expected to incease in the paraganglion in the adrenal medulla. But the satisfactory result was not obtained in this experiment.

To obtain a good result, unilateral adrenal cortex was excised. In this adsenal medulla, conversely, noradrenaline will be expected to increase. But, by the potassium iodate method, no increase of noradrenaline was observed in the adrenal medulla compared with the normal one What is more, the total amount of catecholamines was almost same in both cortex excised and normal adrenal medulla by the chromaffin reaction.

In conclusion, the action of adrenal cortex on the methylation was not observed in this experiment. The methylation of noradrenaline is considered to occur only in the adrenal medulla

Recently, KIRSHNER and GOODALL[19)33)] observed that the adrenal medulla could con-

vert tyrosine to hydroxytyramine, noradrenaline and adrenaline. When uniformly labeled l tyrosine-C^{14} was incubated with slices of adrenal medulla, hydroxytyramine-C^{14}, noradrenaline-C^{14} and adrenaline-C^{14} were isolated by ion exchange chromatography[34]. More recently von EULER and FLODING[16] demonstrated that noradrenaline added to suspensions of medullary homogenates causes a small but significant increase of adrenaline in the absence of added ATP. Since the adrenal medulla is especially rich in ATP, further additions are not required. KELLER et al[30]. have shown that the methyl group of adrenaline can come from methionine in the intact animal.

The paraganglia and the extra-adrenal pheochromocytomas must be investigated here. As previously described, both intra-adrenal and extra-adrenal chromaffin cells are developed from the sympathogonia. The sympathogonia differentiates to the adrenal medulla and at the same time, differentiates to the paraganglia along the aorta and sympathetic trunk.

The medullary tumors are composed of the sympathetic tumor and the pheochromocytoma. In general, the medullary tumors are classified roughly into the neuroblastoma[12][31] and the pheochromocytoma. However, from the viewpoint of catecholamines metabolism, the neuroblastoma and the pheochromocytoma can not be differentiated histochemically.

The pheochromocytomas in many cases originate in the adrenal medulla, but moreover originate in the neck, the thoracic and the other abdominal cavity. According to E. HUNMPHREY[6] (1940), 13 cases out of 103 pheochromocytomas (necropsies in most cases) were extra-adrenal sites. The investigation by J. B. GRAHAM[21] (1951) revealed that 23 cases out of 203 pheochromocytomas were extra-adrenal sites. SHIBUSAWA[49] investigated in full detail on this subject both in foreign and Japanese literatures (Tab. 1).

Table 1 Locations of the extra-adrenal pheochromocytomas.

	foreign literatures 570 cases	Japanese literatures 48 cases
neck	2	0
thoracic cavity	17	0
ventral surface of abdominal aorta	5	0
organs of Zuckerkandl	15	0
perinephritic region	11	3
the other abdominal cavity	2	2
renal pelvis	0	2
bladder wall	3	0
sacral region	1	0
	56 cases	7 cases

(Shibusawa, 1964)

At any rate, about 10% of all pheochromocytomas originae in the extra-adrenal sites.

The diagnosis of a functioning pheochromocytoma is no longer difficult[2]. The first correct preoperative diagnosis was made by PINCOFF[44] in 1929. Since then, many pharmacologic tests have been introduced to aid the clinician in the diagnosis of this condition. The histamine test[46], introduced in 1945, and the regitine test[40], in 1949, have been the most common screening tests for pheochromocytoma in patients with sustained or paroxysmal hypertension. A more direct chemical examination for secretory products

this tumor was carried out. Examination of the plasma or urine for elevated levels of catecholamines is preferred diagnostic test to date. ENGEL and von EULER[13] were the first to demonstrate elevated levels of urinary catecholamines in patients with a pheochromocytoma. More exacting methods are being developed which measure the metabolites[1] of adrenaline and noradrenaline. Metabolic products of these hormones constitute greater than 70% of their urinary excretion.

The pheochromocytomas in the extra-adrenal sites must be diagnosed carefully. The extra-adrenal pheochromocytomas contain more noradrenaline than the medullary ones. Conversely, according to von EULER[17], the pheochromocytomas which were observed much noradrenaline in urine must be doubted the extra-adrenal sites. These facts suggest that the extra-adrenal pheochromocytomas may originate in paraganglia.

The facts mentioned above were thought over from the chemical and histochemical viewpoints. The paraganglia must be studied pathologically here. TOKORO[54] represented diagrammatically the fundamental structure of paraganglion tissue (Fig. 2). They are composed of vascular channel, parenchymatous cells and nerve. In this definition, he included in the paraganglion tissue not only the adrenal medulla and organs of ZUCKERKANDL[58][59] but the carotid body, glomus jugulare, cardioaortic body, coccygeal body and glomic arteriovenous anastomosis. Accordingly, in the term "paraganglioma", pheochromocytoma, glomus tumor, carotid body tumor and alveolar softpart sarcoma are included. The alveolar softpart sarcoma was nominated by STEWART et al.[52] (1952). They reported 12 cases in this name. But this tumor is considered to be the same that was reported in the name of nonchromaffin paraganglioma by SMETANA and SCOTT[50] on 1951. This tumor originates in the subcutaneous tissue or tissue adjacent to the muscle of thigh, or the tissue along the femoral artery. SMETANA and SCOTT reported 14 cases (retroperitoneum 4 cases, neck 2 cases, thigh 8 cases).

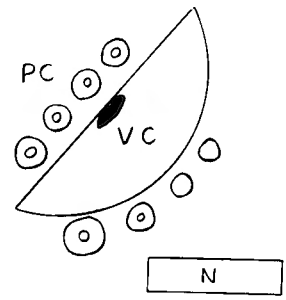

Fig. 2. Diagram of the fundamental structure of the paraganglion tissure.
PC : parenchymatous cell ;
VC : vascular channel ;
N : nerve.

The carotid body[37][38][57] was classified as a "paraganglion" by A. KOHN in the early part of the century because he believed it to be of sympathetic origin and part of the chromaffin system. For this reason, and because the embryology of the carotid body and related structures is still subject to dispute. These organs have been excluded from the term "paraganglion" by HOLLINGSHEAD[26] and LECOMPTE[37][38]. However, the term has continued to appear in papers of GOORMAGHTIGH, PANNIER[20] and WATZKA[55], although sometimes qualified by the prefix "nonchromaffin".

It is now generally agreed that the majority of carotid body tumors[37][38][57] are benign, growing slowly and producing symptoms mainly from pressure on contiguous structures Rarely, such a tumor has been proved to be malignant as evidenced by metastasis to regional lymph nodes or to more remote areas of the body. As described before, the carotid body produces no epinephrine, has no sympathetic nerve supply and is not a endocrine organ. The organ is believed to be a chemoreceptor which responds mainly to

changes in oxygen tension.

Similar bodies have been described near the jugular bulb, the ganglion nodosum of the vagus and the tympanic membrane. Tumors derived from these bodies have more recently been called chemodectomas[42]. However, these tumors including carotid body tumor have been called nonchromaffin paragangliomas by KOHN's pupils.

Generally speaking, the sympathetic paraganglia have chromaffinity and chromaffinity is not observed in the parasympathetic paraganglia. Carotid body tumor, glomus tumor and the majority of softpart paraganglioma, in principle, belong to nonchromaffin group and the medullary tumors and sympathetic paraganglioma belong to the chromaffinoma. After all, the extra-adrenal pheochromocytoma in the clinical sense is considered to be the sympathetic paraganglioma.

SUMMARY

The paraganglia and paraganglioma were investigated histochemically.

(1) Although the paraganglia had been considered to be the storage sites of catecholamines, their secretory activity had not been clarified.

In this report, to prove the secretory activity of paraganglia, dogs were administered Aldomet (L-α-Methyldopa) 500mg orally daily for 20 days. But the histochemical differences were not observed between Aldomet-treated and control groups in both paraganglia and adrenal medulla.

(2) To prove the secretory activity of paraganglia more surely, rabbits were injected reserpine (5mg/kg) intravenously. In all cases, the pronounced loss of catecholamines in the paraganglia was observed. The catecholamines were depleted from the adrenal medulla, either.

The mode of action of reserpine on actecholamines is considered to interfere the amine synthesis. At the same time, the action to the secretory nerve through the central nervous system must be taken into consideration. The two processes: storage and release are presumably balanced in the normal cell. Thus if the storage process is blocked, the result will be a depletion. The secretory activity was proved pharmacologically.

(3) The paraganglia contain noradrenaline chiefly while the adrenal medulla contains adrenaline chiefly. It is generally believed that noradrenaline is the immediate precursor of adrenaline. Accordingly, whether the adrenal cortex may act on the methylation of noradrenaline to adrenaline is subject to debate. The left paraganglion was transplanted in the left adrenal medulla. If the adrenal cortex will act on the methylation, adrenaline will be expected to increase in the paraganglion transplanted in the adrenal medulla. But the satisfactory result was not obtained in this experiment.

(4) To obtain a good result, the unilateral adrenal cortex was excised conversely. In this case, noradrenaline will be expected to increase in the adrenal medulla. But, by the potassium iodate method, no increase of noradrenaline was observed in the adrenal medulla compared with the normal one. What is more, the total amount of catecholamines was almost same in both cortex excised and normal medulla by the chromaffin reaction. The action of adrenal cortex on methylation was not observed. The methylation of noradrenaline is considered to occur only in the adrenal medulla.

(5). The adrenal medulla and paraganglia are developed from the sympathogonia. About 10 % of all pheochromocytomas originate in the extra-adrenal sites. The extra-adrenal pheochromocytoma obtained operatively showed a large quantity of noradrenaline by the potassium iodate method. The extra-adrenal pheochromocytomas contain more noradrenaline than the medullary ones. The paraganglia contain noradrenaline chiefly. These facts suggest that the extra-adrenal pheochromocytomas may originate in paraganglia. Carotid body and glomus jugulare were included in the paraganglia by A. KOHN. Lately they have been excluded from the paraganglia. However, the term has continued to appear in papers from KOHN's lavoratory by his pupils, although sometimes qualified by the prefix "nonchromaffin".

Generally speaking, the sympathetic paraganglia have chromaffinity and chromaffinity is not observed in the parasympathetic paraganglia. Medullary tumors and sympathetic paraganglioma belong to the chromaffinoma. After all, the extra-adrenal pheochromocytoma in clinical sense is considered to be the sympathetic paraganglioma.

The author wishes to express his sincere gratitude to Prof. KIMURA for his helpful suggestion and kind guidance throughout the course of this work and at the same time the author is grateful to Dr. NAKATA and Dr. YOKOTA in our department for their kind heplps.

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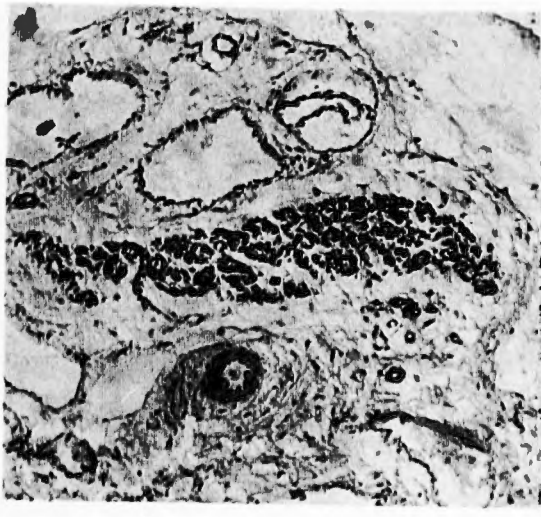

Fig. 3 The paraganglia of rabbit.
The chromaffin reaction. ×100

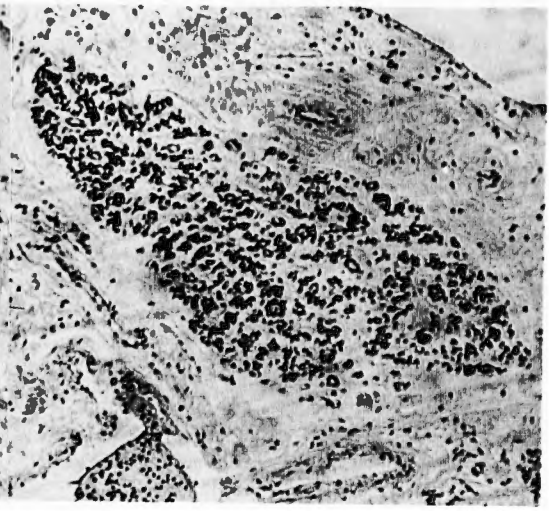

Fig. 4 The paraganglia of rabbit after administration of reserpine.
The chromaffin reaction. ×100

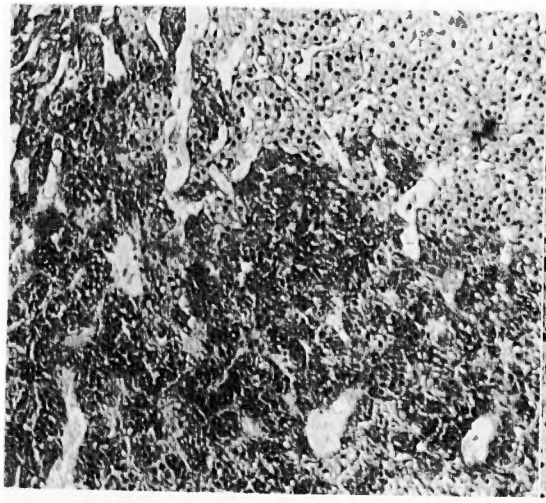

Fig. 5 The adrenal medulla of rabbit.
The chromaffin reaction. ×100

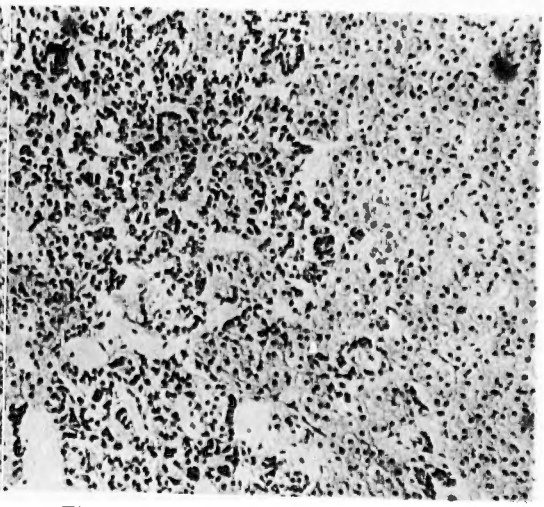

Fig. 6 The adrenal medulla of rabbit after administration of reserpine.
The chromaffin reaction. ×100

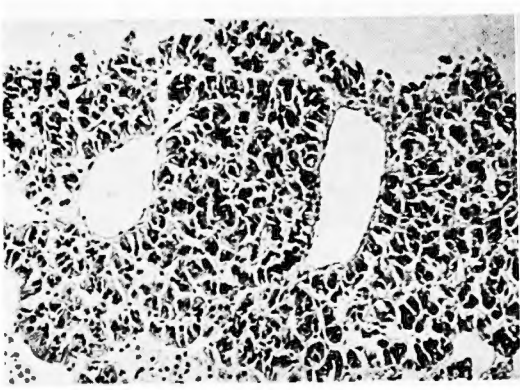

Fig. 7. The cortex excised adrenal medulla of rabbit. The potassium iodate method. ×150

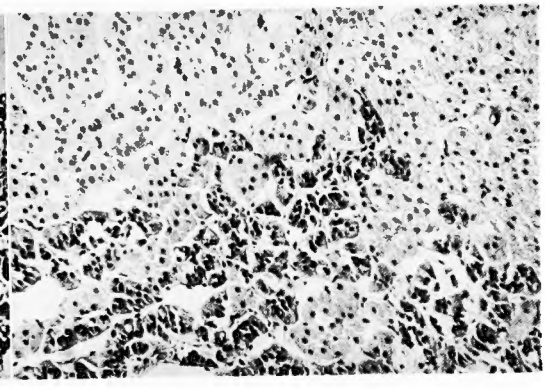

Fig. 8. The normal adrenal medulla of rabbit. The potassium iodate method. ×150

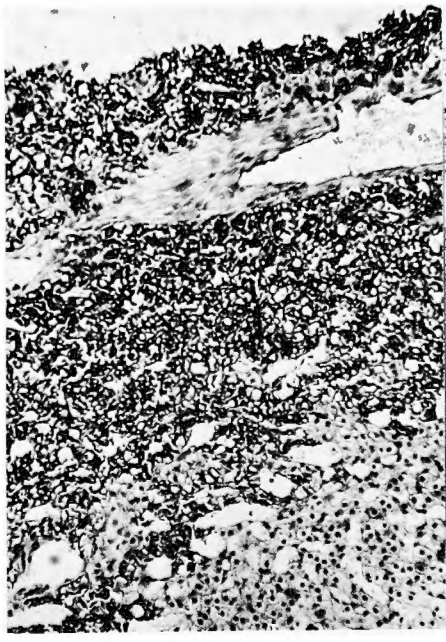

Fig. 9. The cortex excised adrenal medulla of rabbit. The chromaffin reaction. ×150

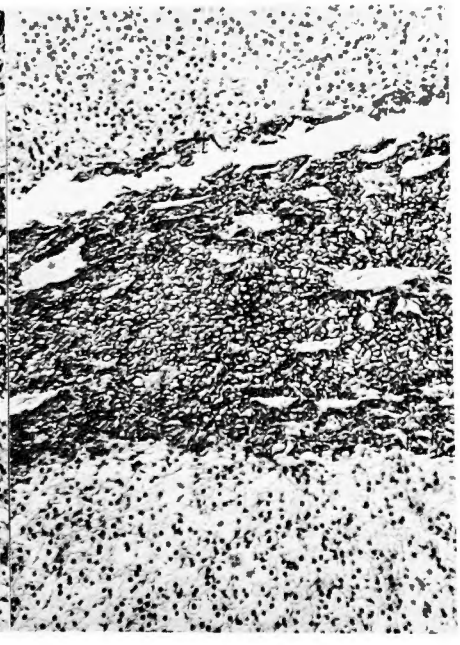

Fig. 10. The normal adrenal medulla of rabbit. The chromaffin reaction. ×150

Fig. 11. Paraganglioma.

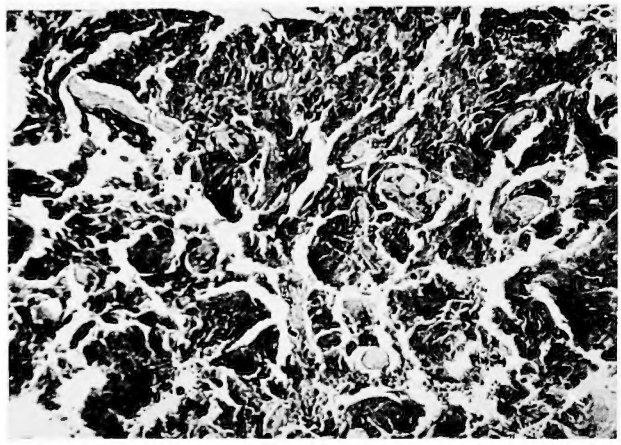

Fig. 12. Microphotograph of Fig. 11.
The potassium iodate method. ×150

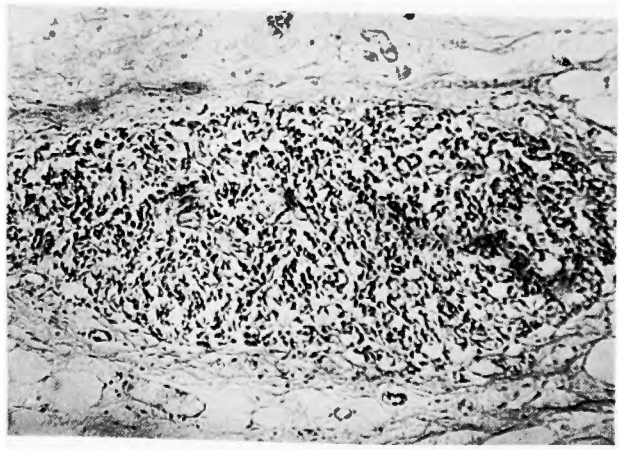

Fig. 13. The paraganglia of man.
The potassium iodate method. ×150

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和文抄録

パラガングリオン及びパラガングリオーマに於けるカテコールアミンの組織化学的研究

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副腎外クロム親和細胞群はKohnによりパラガングリオンと名付けられた．クロム親和細胞はもともと交感神経産生細胞から分化した内分泌性細胞で，交感神経幹内に散在し，交感神経線維とともに副腎内部に侵入して髄質を作り，また交感神経幹及び大動脈に沿つてところどころに群をなしパラガングリオンを作る．

人間に於いては胎児，新生児の“Zuckerkandl器官”として古くから知られており，またこれはすでに幼児期に変性，退化し，成人に於いてもはやパラガングリオンは発見出来ないと一般に考えられて来た．

ところが最近 Coupland により成人に於いてもパラガングリオンは必ず存在することが報告され，また教室の仲田も四肢の末梢性血行障害を有する3例の成人患者から手術的に得られた後腹膜組織内に全例パラガングリオンと認めた．

ここに於いて，副腎髄質及びアドレナリン性神経以外のカテコールアミンの貯蔵，分泌場所として，或いは褐色細胞腫の副腎外の発生母地等としてパラガングリオンについて再検討が加えられるのは意義のあることと思われる．

本編はパラガングリオン及びパラガングリオーマを組織化学的方法により研究した．

(1) パラガングリオンはカテコールアミンの貯蔵，分泌場所と考えられているが，その分泌活動性を証明した報告はない．これを証明する為に犬にアルドメット（L-α-methyldopa）500mgを経口的に20日間投与した．アルドメットは昇圧アミンの生合成の際，脱炭酸酵素の阻害剤として作用すると考えられている．本実験にては，パラガングリオン，副腎髄質ともに対照と較べ，組織化学的差異は認められなかつた．

(2) より確実に分泌活動性を証明する為，更にレセルピン（5mg/kg）を静注した．パラガングリオン，副腎髄質ともに，全例カテコールアミンの著明な消失が認められた．レセルピンは昇圧アミン生合成を阻害するとともに，中枢神経系を介しての作用がある．

本実験により，パラガングリオンの分泌活動性が薬理学的に証明された訳である．

(3) パラガングリオンはノルアドレナリンの含有比が高く，一方副腎髄質はアドレナリンの含有比が高いことが認められている．ノルアドレナリンはアドレナリンの前駆物質であり，従つて，ノルアドレナリンのメチル化に副腎皮質が関与するのではないかとの疑問が起り，種々議論されている．

先つ，左側のパラガングリオンを左側の副腎髄質内に移植した．もし，副腎皮質がメチル化に関与するものであれば，移植されたパラガングリオン中のアドレナリン含有比が増える筈である．然しながら，本実験にては満足すべき結果は得られなかつた．

(4) 逆に，一側の副腎皮質を切除した．この場合は，副腎髄質中のノルアドレナリン含有比は増える筈である．然しながら，クロム親和反応で皮質切除副腎，正常副腎のカテコールアミン総量が殆んど同じであるにかかわらず，沃素酸カリ法により，ノルアドレナリン含有比の増加は認められなかつた．

結局，ノルアドレナリンのメチル化は副腎髄質内でのみ行なわれ，皮質の関与は認められなかつた．

(5) 副腎髄質及びパラガングリオンは共に交感神経産生細胞から分化したものである．全褐色細胞腫の約10%は副腎外のものであることが統計的に調べられている．教室で手術的に得られた副腎外褐色細胞腫についても，沃素酸カリ法により多量のノルアドレナリンを認めた．副腎外褐色細胞腫は髄質性の同腫瘍に較べてノルアドレナリンの含有比が高く，またパラガングリオンもノルアドレナリンの含有比が高い．これらの事実及び発生部位から考えて，副腎外褐色細胞腫にはパラガングリオン起原のもののあることを示唆する．

頸動脈球及びその他の類似構造物はKohnにより，すべてパラガングリオンに含まれたが，最近はパラガングリオンから除外して考える学者が多い．非クロム親和性パラガングリオンと呼ばれることもある．一般に交感神経性パラガングリオンはクロム親和性を有し，副交感神経性のものにはクロム親和性は認められない．

副腎髄質腫瘍及び交感神経性パラガングリオーマはクロム親和細胞腫（Chromaffinoma）であり，臨床的に副腎外褐色細胞腫といわれているものは交感神経性パラガングリオーマと考えてよかろう．