Humoral Regulation on the Functions of Denervated Autologous Free Transplants of the Stomach and the Thyroid Gland

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INTRODUCTION

The functions of the free transplants of various organs might be modified by the operative procedures of denervation, severance of lymphatics, changes in hemodynamics and the immune process. The humoral regulation which is considered to control the functions of denervated free transplants has not yet been clarified. In the present investigations, a portion of the body of canine stomach and the canine thyroid gland were transplanted autologously into the neck and the groin, respectively, using micro-vascular anastomoses to exclude the occurrence of immune process, and humoral regulation on the functions of these denervated transplants were studied.

I. Stomach[27]

Materials and Methods

Adult mongrel dogs of both sexes ranging in weight from 8 to 14 kg were used. Gastric tubes, approximately 10 cm in length and approximately 2 cm in breadth, were constructed from the greater curvature of the stomach preserving the blood vessel supply from the splenic vessels and placed in the subcutaneous pocket at the neck by creating an external fistula. The splenic vessels were anastomosed to the right common carotid artery and the right external jugular vein, respectively, by hand in end-to-end fashion. A Pavlov pouch, which was the same in size as a free gastric tube, was also constructed as a control. The dogs, after fasting for over 24 hours, were anesthetized with intravenous administration of sodium pentobarbital 25 mg per kg. A gummy balloon, 2 cm in diameter and 3 cm in

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length, connected with a polyethylene tube, 3 mm in diameter, was inserted into the lumen of each gastric pouch through the fistula, and intraluminal pressure was recorded with an electric manometer of MP-4T type, amplifier of MP-3A type and polygraph of RM-150 type containing amplifier of RB-2 type and recorder of WI-260 TR type (Nihon Koden Co. Ltd.). The wave patterns of the motility were also recorded after the intravenous injection of synthetic gastrin-like tetrapeptide or serotonin-creatinin-sulphate. The chemical structure of this tetrapeptide is shown to be as follows, and it has been already clarified that the same C-terminal tetrapeptide as this tetrapeptide has all physiological functions of natural gastrin.

X-Try. − Met. − Asp. − Phe. − NH_2, X = H.

Wave patterns were analyzed according to Code's classification[7]. The movement of the gastric pouch was analyzed quantitatively by the "Motility Index". Area "S" obtained by integrating the intraluminal pressure curve, equals the work of gastrointestinal peristalsis and proportional to the momentum during "T" minutes.

$\text{Force}(F) = s \cdot h \cdot A \cdot g$

s : specific gravity, h : height of water column, A : sectional area of water column, g : gravitation.

$\text{Momentum}(M) = f \cdot t = s \cdot h \cdot A \cdot g \cdot t$

t : time

$$\text{Momentum during T minutes} = s \cdot A \cdot g \int_{t=O}^{t=T} h \cdot dt = K \int_{t=O}^{t=T} h \cdot dt$$

"Motility Index" is ratio of "S" to "S_A", which is an area occupied when one centimeter water pressure movement has continued for one minute-a unit of momentum. For actual measurement, the numbers of 1 mm^2 section in the areas S and S_A were calculated.

Gastrin-like tetrapeptide or serotonin-creatinin-sulphate was administered in geometrical series and injected rapidly into the lingual vein and dose-response curves were constructed.

Results

The free transplanted gastric pouch and the Pavlov pouch responded well to synthetic gastrin and serotonin, and showed an increased activity of peristalsis. Dose-response curves were made for the motility of the free gastric transplants and the Pavlov gastric pouch in response to various doses of synthetic gastrin or serotonin. To each dose of the gastrin-like tetrapeptide from 0.01 to 0.25 μg per kg, the free transplanted gastric pouch was more sensitive than the Pavlov pouch. In the former, the motor effect was observed in an extremely low concentration of 0.01 μg per kg, while, in the latter, it was not observed with a lesser dose than 0.05 μg per kg. With each dose from 0.25 to 2.0 μg per kg, the motor effect in the former was lesser than the latter. To each dose of serotonin-creatinin-sulphate from 0.5 to 32.0 μg per kg, the free transplanted gastric pouch was always more sensitive than the Pavlov pouch. In the former, the increased activity was found

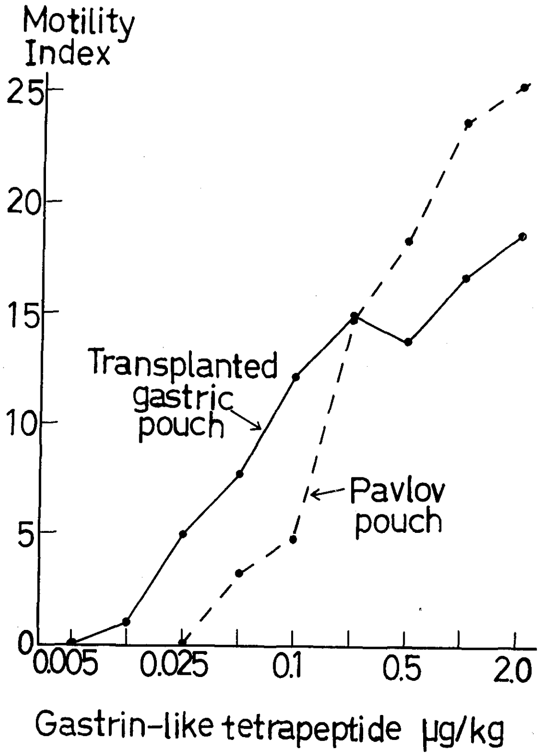

Fig. 1. Dose-response curves for the motility of a free transplanted gastric pouch and a Pavlov pouch in response to gastrin-like tetrapeptide.

after the injection of 1.0 μg per kg, while, in the latter, it was not observed with a lesser dose than 8.0 μg per kg. As shown in these curves, the denervated free gastric transplants responded promptly and in a small amount when compared with the Pavlov pouch (Figs. 1 and 2).

II. Thyroid gland[20]

Materials and Methods

Adult mongrel dogs of both sexes ranging in weight from 6 to 22 kg were used. The animals were anesthetized with intravenous administration of sodium pentobarbital, 25 mg per kg. The right lobe of the thyroid gland was removed preserving the continuity with the common carotid artery and the brachiocephalic vein, in the center of which the entrance of the internal jugular vein is located, and transplanted into the subcutaneous portion of the groin by anastomosing these vessels to the proximal cut ends of the femoral vessels, using the Inokuchi's blood vessel suturing apparatus (Senko Medical Instrument Mfg. Co., Tokyo). The left lobe was left intact as a control.

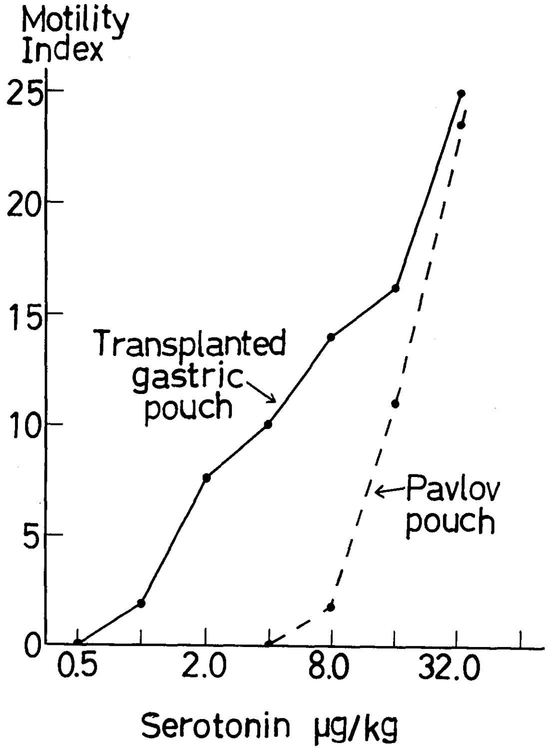

Fig. 2. Dose-response curves for the motility of a free transplanted gastric pouch and a Pavlov pouch in response to serotonin.

1) Forty-eight hours after the intravenous injection of a 50 microcurie dose of ^{131}I, sodium iodide, the dog was sacrificed and the thyroid glands were extirpated, and ^{131}I uptake by thyroid gland was determined. Prior to the injection, the animals were fed with bread and cow's milk or boiled rice and vegetables and white fish for 3 days. 2) The thyroid gland was minced with scissors and homogenized in a glass homogenizer with 0.5 ml of physiologic saline solution. The homogenate, added to 2.5 ml of physiologic saline solution, was centrifuged at 9,500 r.p.m. for 10 minutes at 0°C. The pH of the supernatant fluid was adjusted to 8.6 with dilute 0.2 N NaOH, and the thyroglobulin in the fluid was hydrolyzed with pancreatin at 37°C overnight. Chromatograms were made on paper strips (47 × 5 cm, Toyo No. 50) with a soluvent consisting of the supernatant fluid from a 17 : 2 : 15 mixture of butanol-acetic acid-water, running in a descending fashion in an airtight box. A beaker containing butanol saturated water was placed in the bottom of the box. The resulting chromatograms were dried and placed in an X-ray film holder with a sheet of X-ray film. The film was developed 2 days later. For the quantitative estimation of the ^{131}I of the compounds on the chroma-

tograms, the papers were divided corresponding to the bands observed on the radioautogram, and each section was counted in the well-type scintillation counter. The contents of ^{131}I-compounds: inorganic iodide (I′), monoiodotyrosine (MIT), diiodotyrosine (DIT) and triiodothyronine (T_3) & thyroxine (T_4), were measured. 3) The left lobe of the thyroid was extirpated, and a fasting blood sample was taken for basal protein-bound iodine (PBI) determination. Five units of THYTROPAR (TSH 100 mg, Armour Pharm. Co.) was administered intramuscularly. Blood samples were withdrawn at 3 to 72 hours later. 4) The cranial cervical ganglion of the sympathetic and the nodosal ganglion of the vagus were resected together with the vagosympathetic trunk in the right neck. On the other hand, Evans blue (0.15 ml of 0.5 % solution) was injected beneath the thyroid capsule. A few minutes later the lymph vessels, in which the lymph was stained blue, were ligated, and the lymph nodes which were stained blue were resected. After these procedures ^{131}I uptake by the thyroid gland was determined. 5) The precipitin reaction was attempted between sera of some transplanted dogs and crude saline extracts of normal canine thyroids, according to Ouchterlony's method. 6) The histological examination of the thyroid transplants was also performed. Tissues obtained at autopsy were fixed in 10 % formol, and paraffin sections were prepared and stained with hematoxylin and eosin, or azan stains in the usual manner.

Results

1) The ^{131}I uptake of a thyroid lobe ranged from 3,000 to 12,000 cpm above background which was 1,000 cpm. The transplanted lobe picked up the radioiodine as early as on the 3rd postoperative day. It reached to the normal level on the 13th day, followed by a slight decrease on the 60th day. The ^{131}I uptake of the intact lobe was also suppressed to some extent during early postoperative days. The uptake of the transplanted lobe, when compared with that of the intact lobe of the respective dog, showed a relatively reduced value. The ratio of transplanted/intact of ^{131}I uptake ranged from 0.176 to 1.05. 2) The results of chromatographic analysis of the thyroid gland were expressed as % of total ^{131}I taken into the gland. The ^{131}I remaining at the origin of the chromatogram was excluded from the present analysis, as it was considered to be attributed to the unhydrolyzed ^{131}I-thyroglobulin. The content of inorganic iodide (I′) represented about 15 %, MIT about 30 to 35 %, DIT about 35 to 40 % and T_3 & T_4 about 10 to 20 % of total iodine. When these contents of both lobes were compared with each other, the following tendency could be noted. The content of I′ was almost always lower in the transplanted lobe. The contents of MIT and DIT were higher in the transplanted lobe, and the content of T_3 & T_4 of the transplanted lobe was lower in general, but it could become higher than that of the intact lobe during a period lasting from the 9th to the 13th postoperative day (Fig. 3). 3) After a single injection of 5 units of TSH, the serum PBI level in femoral venous blood increased

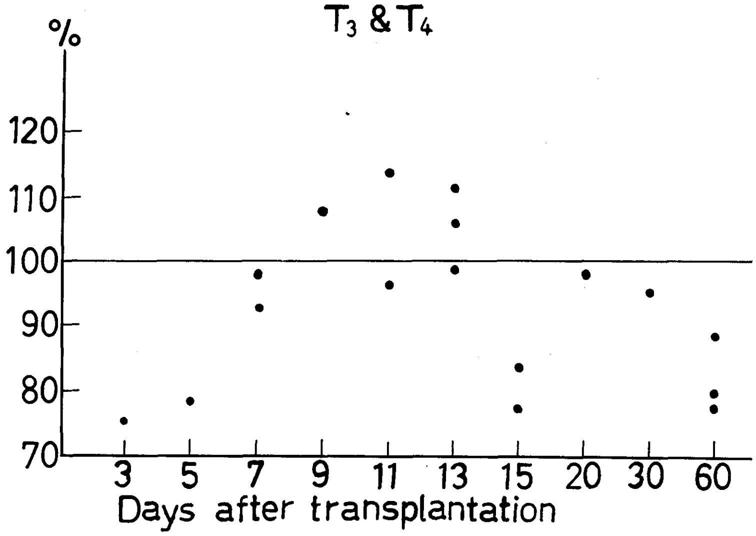

Fig. 3. Comparison of the contents of triiodothyronine and thyroxine in the thyroid gland (transplanted/intact ratio).

from a basal level of 3.04 mcg/100 ml to a peak level of 10.1 mcg/100 ml 3 hours later in a 5-day-old transplant. In the control dogs it took 6 to 12 hours to reach the peak level. In the 74-day-old transplant, the response became somewhat retarded and smaller in amount (Fig. 4). 4) When the unilateral thyroid was denervated by resecting the autonomic ganglia, the ^{131}I uptake decreased in the ipsilateral thyroid lobe. The ^{131}I uptake of the lobe, whose lymphatics were ligated unilaterally did not differ from the contralateral intact lobe on the 5th postoperative day. It was reduced on the 14th day, and became increased on the 22nd day. 5) It failed to demonstrate the auto-antibody against the thyroid tissues in the sera of some transplanted dogs. 6) Histological examination showed no lymphoid cell infiltration in the transplants, but it did show the desquamation of the acinar cells in the early days following transplantation. The damage of the transplants seemed to be the greatest on the 7th and 9th days, and it showed the normal histology on the 13th day.

DISCUSSION

It is well known that gastrin shows several effects on alimentary structures except for gastric secretary stimulation. Hitherto, considerable interest has been shown in the effects of gastrin and gastrin-like peptides on gastrointestinal motility. Blair et al[4]. clarified that an intravenous injection of the extract from the antrum into

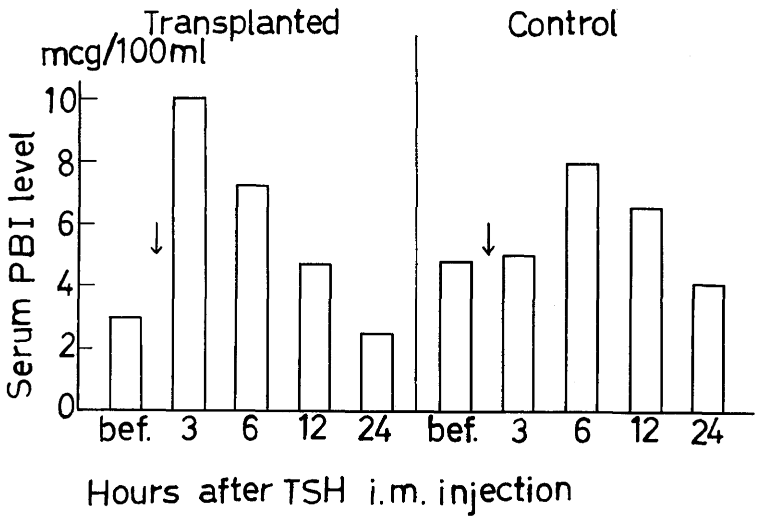

Fig. 4. Effect of TSH on serum PBI level.

anesthetized cats not only stimulated gastric secretion, but also produced s strong "pressor effect" on the stomach and upper small intestine. Gregory and Tracy[11] reported that in the Heidenhain dog pouch a single rapid intravenous injection of purified hog gastrin I and II caused a powerful contraction, followed by a period of rhythmic activity and an increase in tone. Smith and Hogg[24] showed that gastrin II had a readily demonstrable motor effect on the small intestine and colon. Neely[21] showed that in anesthetized cats an approximately equal effect on the small intestine and colon was observed in response to the administration of gastrin, and a lesser on the stomach was observed. Bennett et al.[2] have studied the effects of gastrin and pentagastrin (I.C.I. 50,123) on isolated strips of human gastrointestinal muscle. Both substances caused contractions of gastric muscle, but upper and lower small intestines were unresponsive. Strips from the ascending and sigmoid colon responded with small contractions. Jacoby and Marshall[17] found in anesthetized dogs that gastrin tetra- and pentapeptide and hog gastrin II were a potent stimulant of gastric antral motility, but did not significantly affect circular, duodenal, ileal, or colonic contractile activity. Isenberg and Grossman[16] have demonstrated in dogs that a continuous infusion of porcine gastrin preparations, including crude gastrin, gastrin I or gastrin II, in doses submaximal for gastric acid secretion, stimulated rhythmic antral contractions in the intact stomach or innervated antral pouch of dogs. Kelly[18] reported that gastrin, gastrin pentapeptide and synthetic human gastrin I strikingly increased the frequency of the gastric pacesetter potential and the incidence of bursts of action potentials. However, Sugawara et al.[25] reported that neither intravenous pentagastrin nor endogenous gastrin caused any observable change in gastric tonus, whether in vagally innervated or denervated preparations.

Bennett[1] reported that gastrin acts on the postganglionic parasympathetic nerves and the contraction produced by gastrin is caused by the release of acetylcholine. Bennett et al.[2] also showed that both gastrin and pentagastrin stimulate receptors on or in the smooth muscle cell. Neely[21] observed that gastrin maintained its effect in cats which were atropinized, and moreover were administered with α-, and β-adrenergic blockades, although the effect of gastrin was abolished in only atropinized cats, and stated that gastrin acts without release of acetylcholine. According to Jacoby and Marshall[17], gastrin tetrapeptide does not act directly on smooth muscle, but on nervous elements in the stomach wall. It is well established that truncal vagotomy or vagal denervation of the gastric pouch reduces the responsiveness of the acid and pepsin secreting cells to both gastrin and histamin[9][23]. Vogelfanger et al.[26] have clarified that the significant acid output of a transplanted fundic pouch to psychic stimulation is solely mediated by vagal release of gastrin. Because motor response of the denervated gastric pouch and colon to the tetrapeptide was observed in the Yukimori's experiments[27], it must be considered that gastrin acts on parasympathetic ganglia or postganglionic fibers, or directly on smooth muscles. According to Gregory's view, gastrin may be classified into a transmitter substance[12]. Judging from the experimental results of Bennett and Neely, because premedication of atropine intercepted the effect of gastrin completely or partially, a direct action on smooth muscle is difficult to consider. Therefore, it has been presumed that there is some other factor which mediates between gastrin and smooth muscle. Mikos and Vane[19] reported that hamster stomach and descending rat colon were contracted by doses of gastrin similar to those required by the bullfrog gastric mucosa for its acid secretion. Bennett[3] found that gastrin stimulated human isolated gastric muscle in doses as low as 0.05 μg per ml. Therefore, it has been considered that bowel movement in response to gastrin is of physiological significance.

Judging from the dose-response curve in comparison between a vagally and sympathetically denervated, free transplanted gastric fundic pouch and a vagally and sympathetically innervated Pavlov pouch in our experimental results, the former responded to gastrin-like tetrapeptide in a much lower concentration than the latter. This has indicated that gastrin is a humoral factor with regard to the bowel movement.

Serotonin is released from the gastrointestinal wall by its distention or ischemia. Bülbring et al.[5] indicated that serotonin was released from the mucosa when pressure was applied and that serotonin applied to the mucosal surface of the gut stimulated afferent nerve endings, initiating the peristaltic reflex. Bülbring and Gershon[10] have shown that serotonin could stimulate ganglia in the myenteric plexus. And they have advanced the hypothesis that serotonin, like acetylcholine, participates as a neurotransmitter in the activation of the intrinsic inhibitory ganglion cells of the gut. This hypothesis has not been completely accepted for the following reasons: It is impossible to demonstrate consistent intestinal motor responses from intraluminal instillation of serotonin. It appears that some peristaltic activity can occur in a segment of intestine in which the mucous membrane has been removed. Furthermore, rats depleted of serotonin on tryptophan-free diets continue to demonstrate peristaltic activity. In 1967, Hiatt et al.[13] presented evidence for the existence of a hormone-like substance, Coherin, in the bovine posterior pituitary which exerts a powerful effect on intestinal motility. They showed that marked motor activity could be demonstrated to coincide with the electrical patterns produced by a combination of intraluminal serotonin and intravenous Coherin, and concluded that the interaction of this polypeptide with serotonin in the mucosal receptors is considered to be an essential part of the mechanism of intestinal motor regulation.

In our experimental results, serotonin induced the motor effect in a free transplanted gastric pouch, and it responded more markedly to serotonin than a Pavlov pouch. This has shown that serotonin also possesses the possibility of controlling the motor function of a free transplanted gastric pouch humorally.

Daniel et al.[8] have shown that the concentration of organic radioactive iodine in the lymph draining from the thyroid gland of cats, sheep, rabbits and baboons previously given radioactive iodine is much higher than that in the peripheral or thyroid venous blood plasma and that the administration of TSH increases the concentration still further. The main radioactive iodine component in the lymph draining from the thyroid gland of cats and rabbits was found to be an iodoprotein yielding iodotyrosine upon alkaline hydrolysis. However, the lymphatics play a minor role in carrying away thyroid hormone[22]. On the other hand, changes in blood flow through the thyroid gland do not alter the rate of thyroid hormone secretion[22]. Iino[14] and Ishii[15] observed that the stimulation of the cranial cervical ganglion[14] or the nodosal ganglion[15] caused an increased $PB^{131}I$ level, and concluded that the cervical sympathetic and vagus nerves play an accelerating role in the regulation of the thyroid function.

In our experimental results, the free transplanted thyroid lobes showed relatively low values in both ^{131}I uptake and hormone content (T_3 & T_4) when compared with those of the intact lobe. The content of T_3 & T_4 of the transplanted lobe, which was usually low, became higher than that of the intact lobe during a period lasting from the 9th to 13th postoperative day. Furthermore, in PBI-TSH test, the free transplanted thyroid gland seemed to respond more promptly than the control thyroid gland. These results suggested that the free transplant of the thyroid (where the sympathetic and vagus nerves, both considered as stimulatory to the thyroid function, were resected) showed temporarily an increased sensitivity to humoral stimulation of TSH. The possibility of the occurrence of auto-immune process in the thyroid autotransplantation could be excluded.

Cannon[6] stated that "When in a series of efferent neurons a unit is destroyed, an increased irritability to chemical agents develops in the isolated structures, the effect being maximal in the denervated part." Cannon cited an example that when stimulatory adrenergic fibers degenerate, the dilator muscle of the iris becomes more than normally responsive to adrenalin, and widely dilated, in certain conditions, on the paralyzed side than on the normal side. This was true on the cholinergic nerves. According to this law, the terminal organ, after denervation, acquires the supersensitivity to a chemical transmitter. It is well known that such increased irritability to chemical transmitters has disappeared when the nerves have regenerated.

As shown in our experimental results, the denervated autologous free transplants of the gastric body and the thyroid gland showed temporarily an increased sensitivity to humoral stimulation of synthetic gastrin-like tetrapeptide and serotonin or TSH, respectively. The relationship, which is equal to Cannon's law of autonomic denervation, is assumed to exist between gastrin or serotonin and the free gastric transplants, and between TSH and the free transplanted thyroid gland.

CONCLUSION

Humoral regulation on the functions of the denervated autologous free transplants of the gastric body and the thyroid gland were investigated in dogs. These transplants showed temporarily an increased sensitivity to humoral stimulation of synthetic gastrin-like tetrapeptide and serotonin or TSH, respectively. The relationship, which is equal to Cannon's law of autonomic denervation, is assumed to exist between the hormones and their denervated target organs; for example, between gastrin or serotonin and the free gastric transplants, and between TSH and the free transplanted thyroid gland.

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