STUDIES ON NERVOUS CONTROL OF THE SALT COMPOSITION IN SALIVA

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The investigation on the mechanism of the salt secretion through salivary glands has been performed by many workers since Heidenhein and Babkin first established the fundamental concept of the complicated mechanism of secretory process at the end of the last century. According to their classical experiments, it is quite reasonable to presume a direct effect of serum salt concentration upon salivary composition, since the precursor fluids of saliva are produced from tissue fluid which equilibrates with serum. Nishikawa reported that changes of salt concentration in serum can directly influence salivary composition. By injecting hypertonic NaCl and KCl solution intravenously into dog, he verified that the salt concentrations in saliva, from parotid as well as submaxillary gland, were increased in proportion to the increases of the same salt concentration in serum. Beer and Wilson also reported that the salt concentrations in saliva increased after intravenous injections of CaCl$_2$ and Na$_2$CO$_3$. On the other hand, Yoshimura and his collaborators performed an interesting experiment on this problem. They perfused the submaxillary gland of dog with normal blood using Inoue's perfusion technique and injected hypertonic NaCl solution (10%) into the systemic circulation of the dog from which the blood vessels, running through the submaxillary gland, had been separated. They found that the Na$^+$ and Cl$^-$ concentration in saliva from the perfused gland were elevated in proportion to the increases of the salt concentration in systemic blood. When hypertonic glucose solution (50%) was injected intravenously instead of hypertonic NaCl solution into this dog, similar results were obtained with saliva from the perfused gland, while the Na$^+$ and Cl$^-$ concentration in circulating blood serum were reduced in this case. Moreover, they also pointed out that, by cutting off all the nerves around the perfused glands, such an effect of osmotic changes in systemic blood upon ionic concentrations in saliva was abolished.

From these findings it was concluded that the salt concentrations in saliva were controlled not only directly by their respective ionic concentrations in the

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blood circulating in the gland (direct effect), but also by the nervous influence which reflected from the change in osmotic pressure of systemic blood.

Consequently, it is reasonable to postulate that either some cranial nerves, sympathetic nerves or both transmit the impulse to salivary glands from the osmotic center which is excited by an increase of blood osmotic pressure. Since BABKIN et al. pointed out at the end of the last century that the salivary glands are innervated by the parasympathetic as well as sympathetic division of the autonomic nervous system, it has been generally accepted that the submaxillary gland is innervated by the chorda tympani as well as sympathetic nerve from cervical ganglia. In previous experiments, however, the saliva was obtained by tetanic stimulation of the peripheral cut end of chorda tympani. Therefore, it is impossible to suppose that the chorda tympani transmits this impulse to the salivary gland. In respect of the influence of sympathetic nerve or adrenalin upon the salt concentrations in saliva, LANGSTROTH et al. found and MORI found that the Na⁺ and Cl⁻ concentration in saliva increased after administration of adrenalin. On the contrary, YOSHIMURA et al. reported that, after ipsilateral sympathetic ganglionectomy in the cervical region, the Na⁺ and Cl⁻ concentration in saliva from the ganglionectomized side were elevated. Thus, effects of sympathetic nerves on ionic concentrations in saliva reported in literatures are conflicting.

The author’s present studies were undertaken to determine how the nervous impulse from the center can affect the ionic concentrations in saliva, reflecting from the changes in osmotic pressure in systemic blood. The studies were performed under two categories.

In the first category, the aim of the experiments was directed to the identification of efferent nervous pathways which mediate the impulse from brain to the submaxillary gland, and in the second category, to the elucidation of mechanism by which the salt concentrations in saliva are controled under nervous influences.

**EXPERIMENTAL METHODS**

Target organs were the submaxillary glands of dog. About 100 including male and female mongrel adult dogs, weighing between 8 and 13 kg, were used in the experiments. The animals were anesthetised with intravenous injection of 0.04 cc/kg of Rabonal (2.5% thiopentothal sodium). The saliva was collected through polyethylene cannula (T. Igarashi Co. Ltd., Size No. 15) inserted into WHARTON’s duct. The salivary secretion was provoked, unless otherwise stated, by the stimulation of the peripheral cut end of chorda tympani with square-wave electrical pulses (Nihon-Koden Kogyo Co. Model MSE-20 stimulator) at frequency of 20 c.p.s. and pulse duration 4 msec, in accordance with WILLIS and FUNAKOSHI. The voltage for stimulation was changed from 1 to 10 volts to change the rate of salivary flow. Na⁺ and K⁺ concentration in saliva and blood serum were determined with Lange’s flame photometer and Cl⁻ by BRUN’s modification of SCHALES and SCHALES’ method. Total CO₂ content was
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determined by Kopp-Natelson Microgasometer, model 600.

The animal experiments were divided into two groups according to the aim of experiments, i.e., studies of the efferent nerves which control the salt concentration in saliva, and studies on the mechanism controlling the salt concentration under nervous impulse.

Thus, details of scheme of the experiments will be described under the respective titles as follows:

(1) *Studies on the efferent nervous pathways from the brain to the submaxillary gland.*

As already mentioned in the previous reports\(^6,15\), the salt concentrations in saliva collected from the submaxillary gland perfused with normal blood can be changed, reflecting from the change in osmotic pressure of systemic blood of the dog to which the perfused gland is connected with nerves. Thus the effect of change in the tonicity of systemic blood upon the salt concentration in saliva from the perfused gland was examined in the present experiments after cutting various kinds of efferent nerves which may connect the submaxillary gland to the brain of the dog. In this series of experiments the blood vessels which supply the blood to the submaxillary gland were separated from the systemic circulation on one side of the dog, and the gland was perfused with the normal blood collected from the same dog prior to the experiment, while the gland on the other side remained in situ as the control.

The probable nerves which may transmit the impulse from the brain to the submaxillary gland are sympathetic nerve, glossopharyngeal nerve, vagal nerve and hypoglossal nerve. Some or all of the nerves were cut off near jugular foramen on the side of perfused gland. The ipsilateral sympathetic ganglionectomy was also performed in the cervical region in accordance with the method of Yoshimura, Inoue and Fujimoto\(^11\). After the operation on these efferent nerves, 10% NaCl solution was infused into the systemic circulation (usually 8 cc/kg). Before as well as after infusion of hypertonic NaCl solution, the saliva was collected from both the perfused and control glands by chorda stimulation, and the salt concentrations in the respective saliva were analysed with special reference to the rate of salivary flow.

(2) *Studies on the site of nervous action and the mechanism controlling the salt concentrations in saliva.*

According to the previous report\(^16\) from the author's laboratory there are two possible sites in the gland at which the salt concentration in saliva may be controlled. One is the acinus part and the other is the duct part of the gland. In the present experiment, the role of the duct in controlling the salt concentrations in saliva was examined as the first step. For the purpose, effects of impairing the function of duct with HgCl\(_2\) were studied at first and then the stop-flow analysis was performed with the submaxillary gland. As the next step, the role of the duct in the nervous control of salivary salt concentration was examined by making use of HgCl\(_2\) injection technique and the method of salivary gland perfusion.

(i) *HgCl\(_2\) injection study.* According to Henriques' experiment\(^17\), 0.20 cc of 0.05% HgCl\(_2\) solution was injected retrogradely into the duct of dog's submaxillary gland and was left there for 5-10 minutes. The solution was stained with phenol red. Then the saliva was secreted by the stimulation of chorda tympani, and the collection of the samples was begun after the color of phenol red was cleared up. The histological changes of the salivary gland as a consequence of HgCl\(_2\) injection were microscopically examined after the fixation with Carnor's solution and the usual Hematoxylin-Eosin staining. And the effects of infusing 10% NaCl solution or 2.5% glucose into the systemic circulation was compared between the normal control and the gland pretreated with HgCl\(_2\). Effects of infusing hypotonic glucose solution in a large amount, e.g., 1000 cc/kg, to dilute the blood concentration and to reduce its tonicity were also examined.
Both perfusion and non-perfusion experiments were performed with the submaxillary glands thus pretreated with HgCl₂.

(ii) Stop-flow analysis. After the duct of submaxillary gland was clamped, the tetanic stimulation was applied to the chorda tympani for one minute, and then the clamp was released. The stimulation still continued for another minute, and the saliva from the duct was separately collected every two drops at a time in weighing bottles. The volume of the saliva collected by this procedure was measured gravimetrically by a precision balance. (One drop of saliva corresponds approximately to 0.02 mg.) The collected samples being diluted with 3 cc of distilled water, Na⁺, K⁺ and Cl⁻ concentration of saliva were measured by the methods mentioned above.

According to HENRIQUES17,18) the total volume of saliva which fills one duct space from the opening to the proximal end may amount to about 0.2 ml. Thus, the ionic concentrations in saliva subjected to the duct function can be analysed by this method. And thus, the author conducted stop-flow analysis with one and the same submaxillary gland before and after infusion of hypertonic solution in systemic circulation. A similar comparison was made also with one and the same gland before and after HgCl₂ injection.

EXPERIMENTAL RESULTS

(1) Studies on the efferent nervous pathways from the brain to the submaxillary gland.

In the author's present experiments as well as previous ones6), it was difficult to presume that the chorda tympani can mediate the impulse from the brain to the submaxillary gland, because the saliva was always initiated to secrete by the electrical stimulation of chorda tympani which was separated from the central root. Thus, it seems to be reasonable to suppose that the sympathetic nerve might transmit the impulse to the submaxillary gland. In order to clarify the function of sympathetic nerve, an experiment was performed with a dog of which the total cervical ganglia of sympathetic nerve had been excised on one side of the neck. The submaxillary gland on the side of sympathectomy being artificially perfused with normal blood, the hypertonic NaCl solution was infused into the systemic circulation of the dog, and saliva was collected after chorda stimulation from the perfused gland as well as the non-perfused one at the control side.

The result of this experiment is illustrated in FIG. 1. The solid circles in the figure represent the ionic concentrations in saliva on the side of sympathetic ganglionectomy, while the empty ones are those of the control side. The Na⁺ and Cl⁻ concentration in saliva from the sympathectomized side were always higher than those from the control side. YOSHIMURA et al.11) explained this effect of sympathectomy as due to the loss of tonic inhibitory action of sympathetic nerve upon the Cl⁻ pump of salivary gland. The effect of infusion of the hypertonic NaCl solution was clearly demonstrated in saliva from the sympathectomized side as well as from the control side. In FIG. 1, the vertical columns on the right represent the salt concentrations of blood serum, and its
shaded parts, their increase after the infusion of hypertonic NaCl. The salt concentrations in saliva obtained from the control side after NaCl infusion are indicated by the empty triangles, while those from the sympathectomized side by the solid triangles. The figure illustrates that the Na\(^+\) and Cl\(^-\) concentration in saliva from both glands were raised by similar amounts to those in systemic blood serum, while the K\(^+\) concentration was hardly influenced.

From the result of this experiment, it is clear that the ipsilateral sympathetic ganglionectomy can not abolish the effect of hypertonicity in systemic blood upon salivary salt concentration. Thus, it is concluded that the autonomic nervous fibers by any route other than sympathetic nerve should mediate the impulse transmission from the brain to the submaxillary glands.

Fig. 1. Effect of 10% NaCl infusion upon ionic composition of saliva from perfused gland after sympathectomy.
Sugimoto suggested that any nerves other than the chorda tympani and the sympathetic nerve are involved in the nervous supply to the submaxillary gland, since he ascertained a salivary flow from the submaxillary gland of dog after removal of cervical ganglia and the chorda tympani. Koporow and Emmelin also ascertained that the salivary reflex on the parotid gland of dogs did not disappear after parasympathetic denervation. In anatomical investigations in man, Guerrier and Bolonyi found nerve fibers from a cervical branch of the facial nerve entering the submaxillary gland, and fibers from the hypoglossal nerve which enter the sublingual glands. Thus glossopharyngeal, vagal and hypoglossal nerve are suggested as the possible nerves which transmit the impulse to the submaxillary gland.

In order to determine which nerves can cooperate in transmission of impulse of hypertonicity to the submaxillary gland, the glossopharyngeal nerve, vagal nerve and hypoglossal nerve were cut off on one side of the dog's head.

![Graph](image)

Fig. 2. Effect of 10% NaCl infusion upon ionic composition of saliva from perfused gland after cutting the glossopharyngeal, vagal, and hypoglossal nerve.
and the submaxillary gland on the same side was perfused with its own normal blood, while the gland on the other side was supplied with the normal circulation as the control. Then the hypertonic NaCl solution was infused into the systemic circulation of the dog, and the saliva was collected after chorda stimulation from the submaxillary glands on both sides. The relation between the salt concentrations in saliva and the rate of salivary flow examined in this experiment is illustrated in Fig. 2. The empty circles show the salt concentrations in saliva from the control gland, while the solid circles are those of the saliva from the perfused gland from which the above-mentioned nerves were cut off. The figure clearly demonstrated that the salt concentrations in saliva from the control gland and those from the perfused gland are the same as one another before the infusion of hypertonic NaCl solution. After the infusion, the salt concentration of systemic blood were increased by 20-30 mM, as are shown with shaded columns in Fig. 2, and the Na⁺ and Cl⁻ concentrations in saliva from the control gland were also raised similarly to those of systemic

![Graph showing the effect of 10% NaCl infusion upon ionic composition of saliva from perfused gland after cutting the glossopharyngeal nerve.](image)

**Fig. 3.** Effect of 10% NaCl infusion upon ionic composition of saliva from perfused gland after cutting the glossopharyngeal nerve.
blood serum, while those from the perfused gland remained unchanged. This result is coincident with that of YOSHIMURA et al. which was obtained after cutting off all the nervous supply around the perfused gland.

Consequently, it is presumed that the nerve fibers controlling salt secretion should be involved in the above-mentioned three nerves.

In the next step of experiments, one or two of these three nerves were cut off separately on one side of the dog, and similar perfusion experiments were performed in order to determine which nerve may play the main role in controlling the salt concentrations in saliva from the submaxillary gland of dog.

Fig. 3 illustrates the experimental results obtained after the sectioning of glossopharyngeal nerve. The salivary Na\(^+\) and Cl\(^-\) concentration (solid triangles) from the perfused submaxillary gland on the denervated side were raised as indicated by the solid lines which run at a higher level than the control (dotted) lines obtained before infusion of hypertonic NaCl solution. The rise of

**Fig. 4.** Effect of 10% NaCl infusion upon ionic composition of saliva from perfused gland after cutting the vagal and hypoglossal nerve.
salivary salt concentration was similar to the case of the gland in situ (the control gland). Similar results were obtained after the sectioning of ether vagal or hypoglossal nerve. Thus, when only one of the above mentioned nerves was cut off from the root, it was impossible to abolish the influence of the hypertonicity of systemic blood upon the submaxillary gland via nervous pathway. From these results, similar experiments were performed after cutting two of these three nerves.

Fig. 4 illustrates the result obtained after sectioning both vagal and hypoglossal nerves. The empty points represent the ionic concentration in saliva from the control gland circulated with the systemic blood. The empty triangles represent the ionic concentration in saliva after the NaCl infusion, while the empty circles, those before the infusion. The increase of ionic concentrations in serum of the systemic blood (20-30 mM) after the NaCl infusion is illustrated by the shaded columns on empty columns showing normal concentration on the right in the figure. The real dots, both circles and triangles are the ionic concentrations in saliva from the perfused gland. The figure clearly demonstrated that the concentration of Na\(^+\) and Cl\(^-\) in saliva from the control gland are increased after the NaCl infusion, while the ionic concentration in saliva from the perfused gland showed no difference after the infusion. Thus, the effect of the increase of blood salt concentrations upon the salivary salt concentrations was completely abolished, after cutting off both the vagal nerve and the hypoglossal nerve. On the other hand, after the sectioning of the combination of glossopharyngeal and vagal nerve as well as that of glossopharyngeal and hypoglossal nerve, the effect of the changes in systemic blood concentrations upon those in saliva could not be completely abolished. It follows that the abolishment of the nervous control is incomplete when one of them, i.e., vagal and hypoglossal nerve, is left intact.

The role of chorda tympani on the mechanism regulating salt secretion through the submaxillary gland could not be examined in these experiments, because the chorda tympani was cut off from the root prior to the experiments in order to stimulate it electrically. In an attempt to clarify the role of chorda tympani in the transmission of the impulse from the brain after infusion of hypertonic NaCl solution, the following experiment was performed.

The submaxillary secretion was initiated by the subcutaneous injection of 0.5 cc of 0.1% pilocarpine into dog of which the chorda tympani was left intact and the above-mentioned two nerves (hypoglossal and vagal nerve) were cut off on one side. The submaxillary gland on the denervated side was perfused with normal blood, while the gland on the other side (the control gland) was circulated with the systemic blood. The Na\(^+\) and Cl\(^-\) concentration in saliva collected from this perfused gland as well as from the control gland were increased after the hypertonic NaCl solution was infused into systemic circulation. Thus the chorda tympani may also transmit the impulse from the brain.
to the submaxillary gland to raise the ionic concentrations in saliva after the infusion of hypertonic NaCl solution.

From these findings, it is concluded that the nervous impulse controlling salt concentrations in saliva, which originates from the brain may be transmitted through all these three nerves, i.e., chorda tympani, hypoglossal nerve and vagal nerve. Thus, the complete block of this impulse can be effected only after section of all these nerves.

(2) Studies on the site of nervous action and the mechanism controlling the salt concentrations in saliva.

(a) Studies on the possible roles of the duct in control of salivary salt concentrations.

(i) HgCl₂ injection study. In order to determine the site of nervous action and the mechanism controlling the salt concentration in saliva, effects of HgCl₂ injection into the duct of the gland were studied.

According to microscopic observations, the histological changes in the duct cells of the submaxillary glands resulting from the retrograde injection of 0.2 ml HgCl₂ comprised slight swelling of the cells, blurring of their outline, picnosis and karyolysis of the cell nucleus, while the cells in the acinus part were almost unaffected.

After damaging the duct, the chorda saliva was collected from the submaxillary gland, as stated in experimental methods. The results of analysis of ionic concentration in the chorda saliva, which was collected from the submaxillary gland after damaging the duct, are illustrated in Fig. 5.

The solid circles show the salivary salt concentrations from the HgCl₂ injected gland, while the empty ones, those from the control gland which was left intact on the other side of the same dog. The Na⁺ and Cl⁻ concentration in saliva from the HgCl₂ injected gland are higher than those from the control gland as HENRIQUES¹⁷) pointed out, approaching those in normal blood plasma, which are presented by vertical columns in the figure. Moreover, changes in the Na⁺ and Cl⁻ concentration related to the rate of salivary flow which are presented by the control points, disappear and tend to remain at the respective constant level. The K⁺ concentration in saliva from the HgCl₂ injected gland was lower than that from the control gland, while the total CO₂ content in saliva did not show any appreciable difference between the two.

These results of HgCl₂ injection experiment give evidence to the hypothesis of THYSEN et al.²³,²⁴) who explained the changes in ionic concentration in relation to the rate of salivary flow as due to the ionic reabsorption in the duct, as was pointed out in the previous paper⁶). The results of this experiment also coincide with the report of YOSHIMURA et al.¹⁰) who confirmed that the salt concentrations in saliva taken from the superficial layer of the gland which corresponds to the saliva secreted from the acini, i.e., the precursor saliva, are close to those in serum. The higher concentration of K⁺ in control saliva
suggests that $K^+$ may be added to the saliva when it passes through the intact duct.

(ii) *Experiments of stop-flow analysis.* In order to confirm the results obtained by the HgCl$_2$ injection experiment and to clarify the role of duct cells in the salt secretion, the stop-flow analysis was performed with the saliva collected from the submaxillary gland injected with HgCl$_2$ and the control gland as well.

The results are illustrated in Fig. 6 where the empty circles show the results of the control gland and the solid ones those of the HgCl$_2$ injected gland. The cumulative volume of salivary drops collected is given along the

![Graphs showing ionic concentration of saliva after injection of HgCl$_2$.](image)

**Fig. 5.** Ionic concentration of saliva after injection of HgCl$_2$. 
abscissa, and the ionic concentrations in every two-drop volume of saliva are taken on the ordinate. The first two drops seem to have come from the most distal segment of the duct, and the subsequent samples from the more proximal segment one by one, while the samples which were obtained at the end of this procedure may be from the acinus part of the gland. According to Henriques\textsuperscript{18}, the cumulative volume of saliva which fills the duct from the opening to the proximal end may amount to about 0.2 ml. As the volume of one drop of saliva was 0.025 ml in this experiment, and the amount of one sample was 0.05 ml, the saliva stagnated in the duct during duct occlusion may have been collected in four samples, i.e., from 1st to 4th sample.

As is seen in Fig. 6, the Na\textsuperscript{+} and Cl\textsuperscript{-} concentration in the samples which were stagnated in the duct of the control gland present a remarkable reduction, while K\textsuperscript{+} concentration is increased. On the other hand, the salt concentration in samples from HgCl\textsubscript{2} injected gland remains at about the same level as that in the sample from the acinus part.

These findings well coincide with results of Yoshimura et al.\textsuperscript{16} which indicate that the precursor saliva produced in the acinus part has higher con-

![Fig. 6. Stop flow analysis after injection of HgCl\textsubscript{2}.](image-url)
centrations of Na\(^+\) and Cl\(^-\), and lower concentration of K\(^+\) than those in saliva excreted out of the duct, and thus the ionic composition is close to that in the tissue fluid which equilibrates with the blood serum. From these results, it is concluded that Na\(^+\) and Cl\(^-\) are reabsorbed at some site along the duct, whereas K\(^+\) is secreted, while the precursor saliva is passing through the duct. YOSHIMURA et al.\(^{16}\) maintained that a striated portion is interposed along the duct epithelium and displays the reabsorption of ions from the precursor saliva running through the duct. There are reported many works which support this view. It is not clear, however, from the present experiments whether or not the striated portion may play an important role in the reabsorptive process, and this problem will further be dealt with in future.

(b) Studies on the role of duct in nervous control of salivary salt concentrations.

From the above experiments, it is clear that a site of control of salt concentration in saliva resides in some section along the duct of the submaxillary gland. Thus, the role of the duct in nervous control of salt concentration in saliva was examined in the next step. The experiments were performed in three stages. In the first two stages of experiments, a possible role of duct on the influence of changing salt concentration in systemic blood upon the salivary concentration was examined. In the last stage, the role of duct upon the nervous influence on salt concentration in saliva from the perfused gland was studied. The experimental results will be described in sequence of experiments.

(i) Effect of infusion of 10% NaCl solution into the systemic blood. After the duct of ipsilateral submaxillary gland was damaged by the retrograde injection of HgCl\(_2\), hypertonic NaCl solution was infused into systemic circulation of the dog, and its influence on the ionic concentrations of saliva collected from the damaged gland as well as from the control one were examined.

The results of the experiment are illustrated in Fig. 7, where empty circles represent the concentrations of saliva from the control gland, while the solid circles represent those from the damaged gland. It is indicated in the figure that the Na\(^+\) and Cl\(^-\) concentrations in saliva from the control gland (empty triangles) were elevated after the injection, parallel with the increase of the ionic concentrations in serum which are shown by the shaded columns in the figure. On the other hand, the ionic concentrations in saliva from the damaged gland are maintained at the respective constant level, and are hardly influenced by the NaCl infusion in this case, except a slight rise of Cl\(^-\) concentration after the infusion. In other experiments, however, slight effects similar to Cl\(^-\) were often detected with Na\(^+\).

As to the K\(^+\) concentration and total CO\(_2\) content in saliva, there was no difference between those before and after infusion of 10% NaCl solution respectively with the control gland and the damaged one.

From these results, it is suggested that the duct cells may play the most
important role in reflecting the change of serum salt concentration upon the salivary salt concentration, though a direct effect of rise of salt concentration in serum upon that in saliva cannot be neglected, as was the case in a rise of salivary Cl⁻ concentration.

In an attempt to confirm the above suggestion, the stop-flow analysis before as well as after the infusion was performed with the normal, not damaged submaxillary gland.

The results are illustrated in Fig. 8, where the empty circles represent the ionic concentration of saliva before the NaCl infusion, while the solid circles...
represent those after the infusion. It is demonstrated that the decrease of Na\(^+\) and Cl\(^-\) concentration due to reabsorption through the duct portion is lessened after the infusion of 10% NaCl solution.

It follows that the salt reabsorption through the duct should be inhibited under the influence of increased salt concentration in systemic blood. The opinion that the duct system of salivary glands play the important role in the elaboration of the salivary composition has been confirmed in the past few years by Burgen and his colleagues\(^{25,26}\), and Langley and Brown\(^{27}\).

(ii) Effect of infusing 2.5% glucose solution into the systemic blood after injection of HgCl\(_2\). In the above-mentioned experiments, the role of duct cells after infusion of hypertonic solution into the systemic circulation was experimented. In an attempt to examine the activity of the duct cells, when the tonicity of systemic blood was reduced, a large amount (1000 cc/kg) of 2.5% glucose, instead of hypertonic NaCl, was infused into the systemic circulation of the dog, and the salt concentrations in saliva from the normal gland as well as the HgCl\(_2\) injected gland were determined. The results of the experiment are illustrated.

![Stop-flow analysis of ionic excretion of saliva after 10% NaCl infusion.](image-url)
in Fig. 9.

The empty circles represent the concentrations in saliva from the control gland, while the solid circles represent those from the HgCl₂ injected gland before the infusion. After the infusion of hypotonic glucose, the Na⁺ and Cl⁻ concentrations in serum were reduced as illustrated by the shaded parts of the vertical columns in Fig. 9. The salt concentrations (empty triangles) in saliva from the normal gland were reduced after the infusion, probably due to the decrease of serum salt concentration. While the salivary Na⁺ and Cl⁻ concentrations from HgCl₂ injected gland (solid triangles) were also influenced by the reduction of serum salt concentration, the extent of the influence was not so large as that of the saliva from the control gland. The K⁺ concentration and total CO₂ content were not influenced significantly by infusing the hypertonic solution, as may be the case in the increase of the salt concentration in blood serum.

From this experiment, it is presumed that the duct of the gland may play
an important role in the reflection of salt concentration of the circulating blood upon that in saliva when there is an increase as well as a decrease of blood salt concentration. The effects of changing tonicity of systemic blood upon salt concentration in saliva from the HgCl₂ injected gland indicated that the direct effect of serum salt concentration on that in acinal saliva can also play a role above and beyond the role of duct in reflecting change of tonicity of blood upon that in saliva.

(iii) *May the nervous influence upon the salt concentration in saliva be abolished by damaging the duct epithelium?* By the experiments stated above, it was confirmed that the salivary Na⁺ and Cl⁻ concentrations may change by reflecting the change in salt concentration in systemic blood. The mechanism of the reflection may partly be displayed by the direct effect of changes in blood salt concentrations upon the changes in acinal saliva, and partly by the nervous control from the brain which reacts upon changes in tonicity of systemic blood, as was already pointed out by YOSHIMURA et al.⁶ The experiments described in the previous sections demonstrated that influence of changing salt concentration in blood upon saliva is mainly effected by the activity of duct, and thus suggest that the nervous control of the salt concentration in saliva may also be displayed by nervous influence upon the salt reabsorbing mechanism of the duct. That is to say, the reflection of changes in blood osmotic pressure upon salt concentrations in saliva through nervous control may be effected by the nervous regulation of activity of the duct cells. It follows that the impairment of the duct cells with HgCl₂ should abolish the nervous reflection of blood tonicity upon the salivary salt concentration.

In order to confirm this assumption, the following experiment was performed.

In order to impair the duct epithelium, 0.2 ml of HgCl₂ solution was retrogradely injected into the duct of submaxillary glands on one side. Five to ten minutes later, when enough damage had been done, the solution was washed out, and the submaxillary gland was perfused with normal blood by INOUE's method⁷ on one side, while the gland on the other side was circulated with systemic blood as the control. The saliva was collected from both glands by the electrical stimulation of chorda tympani, and the salt concentrations in saliva from both glands were compared.

The result of this experiment is illustrated in Fig. 10 where the empty circles show the salt concentrations in saliva from the normal intact gland and the solid circles those from the HgCl₂ injected gland. While the Na⁺ and Cl⁻ concentrations in saliva from the control gland present a good correlation with the rate of salivary flow, the dependence of the Na⁺ and Cl⁻ concentrations in saliva from HgCl₂ gland on the rate of salivary flow disappear and the concentrations approach those in normal blood serum, as was already pointed out in Fig. 6. Then, the duct epithelium of the control gland was also
impaired by HgCl$_2$ injection and the hypertonic NaCl solution was infused into systemic blood. The increases of ionic concentration in serum after the infusion are indicated by the shaded columns on the right of FIG. 10, where the ionic concentrations in normal blood are represented by empty columns. The Na$^+$ and Cl$^-$ concentrations in saliva from the control gland (empty triangles) rose to a respective high level and were maintained constant irrespective of the rate of salivary flow. The levels are higher than those of the solid circles, i.e., the ionic concentration of saliva from the gland perfused with the normal blood. The rise of the concentration levels is due to the elevation of serum salt concentration after NaCl infusion. On the other hand, the Na$^+$ and Cl$^-$ concentrations (solid triangles) from the gland perfused with normal blood of which the duct was damaged with HgCl$_2$ did not change at all in spite of the changes in the salt concentrations in systemic blood represented by the shaded columns.

**Fig. 10.** Comparison between salt concentration in saliva from the gland perfused with normal blood and that from the non-perfused gland.
in the figure.

As was verified by YOSHIMURA et al.\textsuperscript{6)}, the ionic concentration in saliva from the submaxillary gland perfused with the normal blood should be elevated by nervous control, reflecting the rise of serum ion concentration in systemic circulation if the duct epithelium remained intact. Thus, it was verified by the present experiment that the nervous influence upon the salt concentration in saliva was abolished by damaging the duct epithelium.

DISCUSSIONS

In these experiments, the author analysed the factors involved in the mechanism of nervous influence upon salivary composition, and verified the important role of the duct epithelium in regulating the salt concentration in saliva.

In an attempt to clarify quantitatively the nervous influence upon the salivary concentration, separately from the direct effect of blood salt concentration upon salivary gland under physiological condition with intact nerves, the following calculations were carried out under the premise that the reflection of ionic concentration in blood upon that in saliva is effected only by two factors, i.e., the direct effect of ionic concentration in blood perfusing in the gland and the nervous influence from the osmoreceptors in the brain.

As the nervous influence was inhibited in the experiments by impairing the duct with HgCl\textsubscript{2}, the regression equations of the correlation between the change in ionic concentration in serum and that in saliva from the HgCl\textsubscript{2} gland may give the direct effect of change in blood concentration upon that in salivary concentration. The regression equations thus calculated from the results of five experiments as was explained in Fig. 7 and 9 are as follows:

\[
\text{Na: } y = 0.49x + 0.46 \quad (1) \\
\text{Cl: } y = 0.34x + 3.16 \quad (2),
\]

where \( y \) represents the change in ionic concentration in saliva, while \( x \) that in serum. The interrelation between \( y \) and \( x \) for the respective ion is presented in Fig. 11. While the intercept constant of the equation (1) is very small, that of the equation (2) can not simply be regarded zero in the scope of experimental error. The cause of this positive intercept constant for Cl\textsuperscript{-} may have some bearing with the excretory mechanism of Cl\textsuperscript{-}, and is the problem in future.

The regression lines between the change of respective ion concentration in blood serum and those in chorda saliva collected from the normal gland in situ were calculated from the results of 16 experiments in which the hypertonic as well as the hypotonic solution was infused into the systemic circulation of the dog, and effects of changing blood salt concentration upon salivary composition were determined. The results of calculation are as follows (see also Fig. 11).
The meanings of y and x are the same as in the equations (1) and (2). The constants in the above equations are somewhat different from those obtained by YOSHIMURA et al.\(^6\) from similar experiments. The differences are not, however, statistically significant, and thus will be neglected in discussions.

These regression equations are presumed to represent the total effect of changes in blood salt concentration upon saliva through nervous control combined with the direct effect.

On the other hand, similar regression lines with the gland which was perfused with normal blood separated from systemic circulation were calculated from the results of 13 experiments, as follows,

\[
\text{Na: } y = 0.60x + 0.65 \quad \ldots (5) \\
\text{Cl: } y = 0.59x + 0.88 \quad \ldots (6).
\]

In these experiments, the salt composition in the blood perfusing through the

\[\begin{align*}
\text{Na: } y &= 0.86x + 1.77 \quad \ldots (3) \\
\text{Cl: } y &= 0.80x + 1.50 \quad \ldots (4)
\end{align*}\]
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The gland was always kept constant and normal, while the osmolarity of systemic blood was changed by infusion of hypertonic or hypotonic solution. Therefore, the above regression lines of perfused gland may be regarded as representing only the effect of nervous influence reflecting the change in osmolarity in systemic blood upon saliva.

The comparison of these three pairs of equations reveals that nervous influences illustrated by the equations (5) and (6) are greater than the direct effect of changes in blood salt concentration represented by the equations (1) and (2), though the differences among the regression coefficients of three sets of equations are not statistically significant. If the total effect of changes in blood salt concentration upon the saliva in situ were composed of only the two factors, i.e., nervous influence and of the direct effect, the direct effect might be predicted by subtracting the nervous influences from the total effect on the normal gland in situ. Thus, the constants in the regression equations with the perfused gland, (3) and (4), were subtracted respectively from the corresponding constants in the equations (5) and (6), for the respective ion, and the following regression equations are obtained.

\[
\text{Na: } y = 0.26x + 1.12 \quad \quad \quad (7)
\]
\[
\text{Cl: } y = 0.21x + 0.26 \quad \quad \quad (8)
\]

A simple glance tells us that equations (7) and (8) are different from equations (1) and (2). This fact suggests a doubt as to whether the premise for the calculation might be wrong or the differences be within the scope of experimental error.

Studying the parotid response to Na⁺ depletion in sheep, DENTON et al.\textsuperscript{28} pointed out that there are two major components in the mechanism of parotid response to Na⁺ depletion in normal animal, viz., an increased secretion of mineral corticoid and local parotid factor associated with the decrease of secretion rate. The present experiments with dog’s submaxillary gland revealed that the local factor of the gland pointed out by DENTON et al.\textsuperscript{28} is effected by reabsorption of ions in the duct, and that the salt concentration in saliva is influenced by the direct effect of changes of salt concentration in blood perfusing the gland, and of the nervous control on the reabsorptive mechanism of the duct epithelium of the gland. In the above calculation of the effects of changes in blood ionic concentration, however, the hormonal factor pointed out by DENTON et al.\textsuperscript{28} was neglected. Statistic calculation revealed that the differences of the regression coefficients in the equations (7) and (8) from those in (1) and (2) are statistically insignificant. Thus, the experimental errors are too large for quantitative treatment. It follows that the complete quantitative analysis of factors influencing the ionic composition in saliva is the problem for future studies.
SUMMARY

The present experiments were performed with dog's submaxillary gland, (a) for the identification of efferent nervous pathways which mediate the impulse from brain to the submaxillary gland in reflecting the blood tonicity upon salivary salt concentration, and (b) for the elucidation of the mechanism involved therein. The results are as follows:

1) It was confirmed that the reflection of changes in tonicity of blood upon salivary salt concentrations are effected not only by the direct effect of blood circulating in the gland, but also by the nervous control from the osmoreceptor in the brain. The present experiments revealed that this impulse from the brain is transmitted through three nerves, i.e., chorda tympani, vagal, and hypoglossal nerve.

2) The function of duct cells being impaired by HgCl₂ injection, the intraglandular activities involved in the mechanism of salt secretion was analysed. The saliva collected from the gland impaired by HgCl₂, presented higher ionic concentration than that from the control gland and the concentration was maintained constant irrespective of variation of rate of salivary flow. It is inferred that the saliva thus collected from the gland treated with HgCl₂ is the precursor saliva secreted from the acinus and the experimental verification by the previous authors⁶) that the dependence of the ionic concentration on the rate of salivary flow is effected by the activity of the duct was confirmed. It was verified by the stop-flow analysis that the duct epithelium displays the reabsorption of Na⁺ and Cl⁻ and the secretion of K⁺ in precursor saliva.

3) It was clarified by experiments with the HgCl₂ gland that the nervous impulse mentioned in (1) affects the reabsorption of Na⁺ and Cl⁻ from the precursor saliva running through the duct.

4) The factors involved in the mechanism reflecting the change in blood salt concentration upon salivary composition were discussed and their quantitative analysis was attempted.

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