An Experimental Physiological Study on Plasmin Substances in Saliva of Rabbits

by

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Introduction

It was in the year 1893 that DASTRE[1] gave the term fibrinolysis to the phenomenon that a cluster of fibrins in the blood solves itself without the bacterial contamination and regains the character of fluidity in it.

Much later, in 1946 MACFARLAN et al. [2] found as plasmin a proteolytic enzyme which is concerned in this phenomenon.

Since this time, plasmin and a series of substances related to it are called plasmin substances and till about 1950 these substances had been considered as being involved in shock or allergic complaints through the researches by NOLF[3] and MACFARLAN et al. [4].

In 1952, RATNOFF[5] and STEFANINI[6] among others gave a report that in such clinical instances as a heavy hemorrhage attendant on a surgical operation or purpura a high degree of plasmin activity is found upon a careful examination of blood.

Since 1952 to the present, many researches have been conducted on plasmin substances as having important bearings on hemorrhage and with the discovery of an anti-plasmin agent (epsilon amino-caproic acid) by OKAMOTO et al. [7], interest in plasmin substances came to assume an intensified tone. These plasmin substances exist as an inactive state of plasminogen and of proactivator not only in blood and body liquids, but also in such tissues susceptible of bleeding as the prostate, uterus, thyroid gland, suprarenal body, lymphatic gland, etc. At the time of bleeding, the existence of tissue activator is known to gain in force to a considerable degree.

Confining our attention to the literature dealing with plasmin substances in the oral area, we learn that ALBRECHTSEN et al. [8] had already discovered the activator, TAYLOR et al. [9] denied the existence of plasminogen as well as activator in saliva and the present authors and his collaborator[10], on the other hand, established the existence of three plasmin substances: plasmin, activator and proactivator in human saliva by the use of fibrin plate.

From the fact that fibrinolytic substance exists in human saliva, OSHIKANE[11] has published a conclusion that as saliva promotes the fibrinolysis, wounds inflicted in the oral cavity tend to be healed quicker as compared with those on the other part of human body.

In the First Part of these studies, the authors reported on his findings of plasmin substances in the oral cavity and other related tissues[14]. Here he is concerned with

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plasmin substances found in rabbits from the standpoint of experimental physiology under various conditions as follows.

- 1) *Plasmin substances in the parotid and mixed salivas in rabbits.*
- 2) *Plasmin substances in the circulatory blood and in mixed saliva at a high body temperature.*
- 3) *Plasmin substances to be found in the blood, parotid and mixed salivas through the administration of Mitomycin C.*
- 4) *Plasmin substances to be found in the blood, parotid and mixed salivas through the administration of Streptokinase.*
- 5) *Plasmin substances in the blood at the time of tooth extraction.*
- 6) *Plasmin substances in soft tissues in the oral area.*

The present paper gives some of the salient findings obtained under each of these conditions, with a view to contributing further to the exact understanding of these important substances.

Materials and Method

1. *Experimental animals.*

For the experimental purposes, use was made of male rabbits weighing from 2.5 to 3.0 kg.

2. *Collection of saliva.*

Following a method of salivary collection devised by YAZAKI [12], the authors made a surgical incision on the rabbit and inserted a thin polyethylen tube into the right parotid duct and collected reflex saliva through it by injecting pilocarpine hydrochloride into vena auricularis. For the collection of mixed reflex saliva, the oral cavity was thoroughly cleaned by the use of a saline solution and, after the passage of time, saliva was received in a beaker directly from the mouth.

3. *Thermal box.*

A thermal box designed to increase body temperature of the rabbit was specially devised by HONZAWA of our Department of Physiology. It was 500×500×500 mm in size and was heated by 2 thermal bulbs, 60 V/100 W each, with provision to install a shelf to hold rabbits. This thermal box has the capacity to increase an inside temperature up to 47°C. The rabbits were first taken their normal temperature (via rectum) and then were subjected to various degrees of temperature ranging from 38.5°C to 45.0°C. Six points were arbitrarily selected within this temperature range and salivary secretion was measured and collected at each point in the same manner described above.

4. *Mitomycin C.*

Mitomycin C (MMC) used for the present study was manufactured by Kyowa Hakko Company which was in the state of crystals. This drug was solved in an isotonic salt solution and employed as an injection. Collection of the blood, parotid and mixed salivas was performed in the same manner as before.

5. *Activation of streptokinase.*

For the activation of streptokinase (S. K.) in rabbits, a mixture was prepared by solving varidase (Lederle Lab., American Cynamid Co.) (streptokinase 100,000 u and streptodornase 25,000 u/vial) in 4.0 ml of the isotonic salt solution so that the whole

preparation might be rendered 10,000 u/kg. The mixture thus prepared was further added to 5.0 ml of human serum and it was injected into the experimental animals.

6. *The extraction of teeth.*

The rabbits were extracted of their 4 incisors under no anesthesia. The collection of their blood was made in 5 phases: immediately after, 10 minutes, 20 minutes, 30 minutes and 40 minutes after the extraction respectively.

7. *The extraction of Plasmin substances in the oral soft tissues.*

For this purpose, a modification was used of a method devised by ASTRUP et al. [13]. In the first place, such oral soft tissues as salivary glands (parotid and submandibular glands), lingual, buccal and soft palate mucosae, gingiva and nasal mucosa were removed from these spots fresh in the amount of 1.0 g each. They were repeatedly rinsed in running water. After water was removed by the use of filter-paper and blood as well, these tissues were thoroughly mixed by mortar and pestle. Subsequently, the mass was divided into halves and each was placed in a flash-mixer of 4.0 ml content, to be shaken together with 2 M KSCN solution or 2 M KCL solution serving as extracting agents. When the mixture had been cooled for an hour, it was centrifugally settled at $5,350 \times g$ for 5 minutes to use a supernatant liquid. 0.03 ml of this supernatant liquid was dropped on a plate for the detection of plasmin substances in these tissues.

8. *The preparation of euglobulin fraction of the blood.*

The blood which had been earlier collected from vena auricularis in rabbits was dropped on a fibrin plate as euglobulin fraction for the measurement of plasmin substances. Euglobulin fraction used here was prepared in the following manner. Rabbit blood was diluted by pure water to 20 times the original volume and after its pH was maintained at 5.2 by the addition of 1% acetic acid, it was cooled for 30 minutes and subjected to centrifugal processing at 3,000 r.p.m. for 10 minutes. Its sediment was then dissolved in a borate saline buffer which had the volume corresponding to the volume of the original serum. Buffer solution consisted of two kinds. One was 0.05 M sodium borate solution (19.108 gm. $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ /1 distilled water) and the other 0.2 M boric acid-salt solution (12.404 gm. H_3BO_3 , 2.925 gm NaCl /1 distilled water). One was mixed with the other in the ratio of 1 : 4, with its pH level being maintained at 7.8 by means of a borate saline buffer.

9. *Measurement of euglobulin fraction of the blood and plasmin substances in parotid and mixed salivas as well as in oral soft tissues.*

For the measurement purposes, a modification was used of a method devised by ASTRUP et al. [13], in which the fibrin plate method was adopted. 0.03 ml of each testing material was dropped on a plate and was left standing for 40 hours at a constant temperature of 37°C. The diameters (longest and shortest) of 3 dissolution rings which occurred in consequence was measured in millimeters, the mean of their products being taken as indicative of the degree of activation.

In order to prepare a fibrin plate, 10 ml of 0.6% fibrinogen fluid was taken in a flat Petri dish, 9 cm in diameter, to which was added 0.5 ml of 20 u/ml thrombin (Mochida Seiyaku Co.) solution with a minimum loss of time. After it was exposed to room temperature for an hour, it was used as standard fibrin plate. On the other hand, this standard fibrin plate was further heated for 30 minutes at a temperature of $85^\circ\text{C} \pm 5^\circ\text{C}$ to make a heated plate [20].

10. *The detection of plasmin substances by the use of fibrin plate method.*

For the detailed procedures involved in the detection of plasmin substances by the use of fibrin plate method, reference is made to Part 1 of these studies[14].

Results

1. *Plasmin substances in parotid and mixed salivas in rabbits.*

10 male rabbits were given a surgical operation to incise part of their right cheek region as described in the foregoing section. After their incision, rabbits were kept quiet on a fastening shelf for an hour and parotid saliva as well as mixed saliva was collected by injecting 0.04 ml of 1 % pilocarpine hydrochloride into the vena auricularis. 0.03 ml of saliva thus obtained was dropped on a fibrin plate.

For the preparation of a saliva-S.K. mixture, 25,000 u/ml was added in the same volume as that of a testing material so as to use it for the detection of proactivator.

As given on Table 1, the existence of proactivator was attested to in all the specimens of rabbit parotid and mixed salivas but there were not detected plasmin and activator actions in them.

TABLE 1
Plasmin substances in mixed and parotid salivas in rabbits

Fibrin plate Rabbit No.	Mixed saliva		Mixed+S. K.		Parotid saliva		Parotid+S. K.	
	Heated	Standard	Heated	Standard	Heated	Standard	Heated	Standard
1	—	—	—	49	—	—	—	49
2	—	—	—	64	—	—	—	49
3	—	—	—	56	—	—	—	49
4	—	—	—	72	—	—	—	64
5	—	—	—	56	—	—	—	49
6	—	—	—	49	—	—	—	36
7	—	—	—	56	—	—	—	56
8	—	—	—	63	—	—	—	56
9	—	—	—	49	—	—	—	49
10	—	—	—	56	—	—	—	54

Unit=mm². S. K.=Streptokinase.

2. *Plasmin substances in the blood and mixed saliva at a higher body temperature.*

As described in 3) of Materials and Method above, animals were fastened in a specially designed thermal box in which they were gradually warmed up to 45°C for 3 to 4 hours. Their mixed saliva and circulatory blood were at the same time collected at 6 different thermal points. Some of rabbits were discarded as unfit, for they gave rise to abnormal findings when their temperature exceeded 40°C. When animals were found to be abnormal because of a high fever, they produced an excessively large amount of salivary secretion. For the collection of mixed saliva, 0.04 ml of 1 % pilocarpine hydrochloride was injected into them via vena auricularis at their normal body tempera-

ture and 0.03 ml of reflex mixed saliva thus induced was used for the purpose of plasmin detection.

As seen from Table 2, plasmin activity began to take place in common with the circulatory blood and saliva when their temperature (measured via rectum) reached the neighborhood of 41°C. The existence of activator action was recognized in mixed saliva, while in the euglobulin fraction of blood the existence of plasmin was traced, though in a minute amount.

TABLE 2
Plasmin substances in mixed salivas and blood of rabbits at a
higher body temperature

Rabbit No.	No. 1		No. 2		No. 3		No. 4	
t.m. Rectum temp.	Mixed	Eug.	Mixed	Eug.	Mixed	Eug.	Mixed	Eug.
38.5°C	←==	==	←==	==	←==	==	←==	==
39.0°C								
39.5°C								
40.0°C	←==	==	←==	==	←==	==	←==	==
40.5°C								
41.0°C	←==	==	←==	25 25	←== 16	16 25	←==	==
41.5°C								
42.0°C	←== 42	25 25	←== 16	36 25	←== 25	25 25	←== 16	25 25
42.5°C								
43.0°C	←== 36	36 25	←== 25	25 36	←== 16	16 25	←== 16	36 25
43.5°C								
44.0°C	←== 25	49 25	←== 16	25 36	←== 25	25 25	←== 25	26 46
44.5°C								
45.0°C	←==		←==		←==		←==	

t.m. = test materials. Mixed = mixed saliva. Eug. = Euglobulin fraction of the blood.

←== Top end of the arrow indicates the result of heated fibrin plate, while bottom gives that of standard plate.

← Body temperature at death.

3. *Plasmin activation in the blood, parotid and mixed salivas through the administration of Mitomycin C.*

OKAMOTO et al. [15] made clear the fact that plasmin substance in man and other mammals can be activated through the administration of Mitomycin C, an anti-tumor agent, dosed for a repeated period of time. This fact has been already endorsed by such researchers as OKAMOTO et al. [15] in Japan and LEWIS and FERGUSON [16] abroad. Here the authors were concerned with the use of fibrin plate to ascertain whether plasmin substances would occur in saliva by the simultaneous measurement of euglobulin fraction of the blood as well as mixed and parotid salivas. Results are given in Tables 3 and 4.

Mitomycin C was dosed to rabbits via vena auricularis on a daily basis in the amount of 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg and 3.0 mg/kg respectively. Of these varying dosages, 0.5 mg/kg and 1.0 mg/kg were dosed for 9 days while 1.5 mg/kg and 3.0 mg/kg

TABLE 3
Activation of plasmin substances in parotid and mixed salivas and blood through the administration of MMC

Day	Rabbit No.	No. 1			No. 2			No. 3		
	Dosage	0.5 mg/kg			0.5 mg/kg			1.0 mg/kg		
	t.m. Fibrin plate	Parot.	Mixed	Eug.	Parot.	Mixed	Eug.	Parot.	Mixed	Eug.
1	Heated	—	—	—	—	—	—	—	—	—
	Standard	—	—	—	—	—	—	—	—	—
2	Heated	—	—	—	—	—	—	—	—	—
	Standard	—	—	—	—	—	—	—	—	—
3	Heated	—	—	—	—	—	—	—	—	—
	Standard	—	—	—	—	—	—	—	—	—
4	Heated	—	—	—	—	—	—	—	—	—
	Standard	—	—	—	—	—	—	—	—	—
5	Heated	—	—	16	—	—	25	—	—	16
	Standard	—	—	25	—	36	25	—	16	25
6	Heated	—	—	16	—	—	36	—	—	16
	Standard	—	—	36	—	49	25	—	25	25
7	Heated	—	—	9	—	—	25	—	—	25
	Standard	—	—	16	—	36	25	—	36	25
8	Heated	—	—	25	—	—	16	—	—	25
	Standard	—	—	25	—	36	25	—	42	25
9	Heated	—	—	16	—	—	16	—	—	16
	Standard	—	—	25	—	49	49	—	36	25

MMC=Mitomycin C. Mixed=Mixed saliva. Parot.=Parotid saliva.
Eug.=Euglobulin fraction.

TABLE 4
Activation of plasmin substances in parotid and mixed salivas and blood
through the administration of MMC in large dosage

Day	Rabbit No.	No. 1			No. 2			No. 3		
	Dosage	1.5 mg/kg			3.0 mg/kg			3.0 mg/kg		
	t.m. Fibrin plate	Prot.	Mixed	Eug.	parot.	Mixed	Eug.	Parot.	Mixed	Eug.
1	Heated	—	—	—	—	—	—	—	—	—
	Standard	—	—	—	—	—	—	—	—	—
2	Heated	—	—	—	—	—	36	—	—	25
	Standard	—	—	—	—	—	72	—	—	36
3	Heated	—	—	25	—	—	36	—	—	25
	Standard	—	—	25	36	49	42	25	42	42
4	Heated	—	—	16	—	—	42	—	—	36
	Standard	—	—	25	25	42	46	16	36	42

MMC=Mitomycin C. t.m.=test materials. Mixed=mixed saliva. Parot.=parotid saliva.
Eug.=Euglobulin fraction.

were administered for 3 days, the circulatory blood, parotid and mixed salivas being collected in the same manner as described above.

As seen from Table 3, as regards the administration of 0.5 mg/kg plasmin activity took place in blood after 5 days and activator in mixed saliva also after 5 days. More or less the similar finding was registered in the case of 1.0 mg/kg dosage (No. 3 rabbit). As given in Table 4, the injection of a larger Mitomycin dosage in a comparatively short period of time gave an activation of the blood plasmin in only one case (No. 1 rabbit). Although activator action came to be found in parotid and mixed salivas collected from No. 2 and No. 3 rabbits in 3 days of the administration, yet the existence of plasmin in either case failed to be attested to.

4. *The activation of plasmin substances in the blood, parotid and mixed salivas through the dosage of streptokinase.*

It has been discovered by OKAMOTO et al. [17] that since the blood of dogs or rabbits merely responds to streptokinase very weakly, the addition of human serum in a minute quantity enhances its response anywhere from 100 to 1,000 times. This phenomenon may be accounted for by the fact that human proactivator which is sensitive to streptokinase will activate plasminogen in the canine or leporine blood.

By injecting a mixture of 5.0 ml human serum and 10,000 u/kg streptokinase that was previously prepared into the rabbits via vena auricularis, a temporary plasmin activation was induced in them and, while in this state, measurements were taken of an euglobulin fraction of the blood, plasmin activity in both parotid and mixed salivas secreted by the use of pilocarpine hydrochloride stimulus. These results are given by Table 5.

Immediately after the injection of a serum-streptokinase mixture, the blood registered the strongest activation as much as 225 and then gradual decrease in the volume in

TABLE 5
Plasmin substances in the blood, parotid and mixed salivas in
rabbits through S. K. activation

Rabbit No.	No. 1		No. 2		No. 3	
Fibrin plate	Heated	Standard	Heated	Standard	Heated	Standard
Pre-injection of S. K.						
Eug.	—	—	—	—	—	—
S. K. activation	(169)	(169)	(156)	(169)	(144)	(156)
Parotid saliva	—	—	—	—	—	—
S. K. activation	(49)	(46)	(36)	(49)	(49)	(46)
Mixed saliva	—	—	—	—	—	—
S. K. activation	(53)	(49)	(46)	(49)	(64)	(72)
S. K. injection (3 min.)						
Eug.	64	225	56	210	49	222
Parotid saliva	—	—	—	—	—	—
Mixed saliva	—	—	—	—	—	—
S. K. injection (20 min.)						
Eug.	49	144	46	110	46	132
Parotid saliva	—	—	—	—	—	—
Mixed saliva	—	—	—	—	—	—
S. K. injection (40 min.)						
Eug.	49	100	42	95	46	110
Parotid saliva	—	—	—	—	—	—
Mixed saliva	—	—	—	—	—	—
S. K. injection (2 hrs.)						
Eug.	—	—	—	—	—	—
Parotid saliva	—	—	—	—	—	—
Mixed saliva	—	—	—	—	—	—

Unit=mm. Eug.=Euglobulin fraction of the blood.

S. K. activation=25,000 u/ml of S. K. was rendered as the same volume of test material.

proportion to the passage of time, its plasmin activity entirely disappeared by the end of 2 hours. As shown by the Table 5 saliva specimens failed to register any plasmin activity.

5. *Plasmin substances attendant upon the extraction of teeth.*

Ever since it was made clear in 1937 by MACFARLANE[19] that a surgical invasion would increase plasmin activity in the tissue involved, this theory has found a wide circle of supporters.

The author used the extraction of teeth in rabbits as an instance of a surgical invasion. Incisors were extracted by the routine method and 5 time-points were selected from the extracting operation to the passage of 40 minutes, at each of which the blood was collected via vena auricularis. Results appear in Table 6.

As is learned from it, all the 5 blood specimens attested to the fact of plasmin

TABLE 6
Plasmin substances in the blood in rabbit in the extraction of teeth

Rabbit No.	No. 1		No. 2		No. 3	
Fibrin plate	Heated	Standard	Heated	Standard	Heated	Standard
Dre-extraction (S. K. activation)	— (169)	— (169)	— (169)	— (169)	— (169)	— (169)
Immediately after extraction	64	36	30	56	49	64
After 10 minutes	25	25	—	—	—	—
After 20 minutes	—	—	—	—	—	—
After 30 minutes	—	—	—	—	—	—
After 40 minutes	—	—	—	—	—	—

activity though it was by no means pronounced.

6. *Plasmin substances contained in the oral soft tissues.*

As previously reported[14], the existence of plasmin substances as tissue activator in various soft tissues of the human oral cavity has been endorsed by the present authors, though different in degree. In the same report, he also gave his findings that as an extracting agent 2 M KSCN solution was more advantageous than 2 M KCl solution.

Here, similarly two extracting agents were employed for extracting plasmin substances from the oral soft tissues in rabbits.

Results are given in Table 7.

In the case of 2 M KSCN solution as an extracting agent, all the tissues proved negative, while with the use of 2 M KCl solution the existence of proactivator action was known in the gingiva, palatal and nasal mucosae, activator also existing in the gingiva.

TABLE 7
Plasmin substances contained in the soft tissues of the oral cavity

Fibrin plate	2 M KSCN solution				2 M KCl solution			
			+SK				+SK	
	Heated	Standard	Heated	Standard	Heated	Standard	Heated	Standard
Parotid gland	—	—	—	—	—	—	—	—
Submandibular gland	—	—	—	—	—	—	—	—
Lingual mucosa	—	—	—	—	—	—	—	—
Buccal mucosa	—	—	—	—	—	—	—	—
palatal mucosa	—	—	—	—	—	—	—	16
Gingiva	—	—	—	—	—	16	—	25
Nasal mucosa	—	—	—	—	—	—	—	64

+SK=25,000 u/ml was prepared to make up the same volume as that of testing material.

Considerations

Studies on plasmin substances have been chiefly conducted from a pathophysiological point of view in the human organs and tissues such as the blood, urine, prostate, uterus, thyroid gland disturbance, etc. Mass of knowledge accumulated in this field in recent years, particularly that of fibrinolysis, has attained to the dignity of a distinct area of research in medical sciences.

The latest endeavors are concerned with basic experiments on plasmin substances with the use of dogs and rabbits in order to approximate the results to those of humans. OKAMOTO et al. [17, 18] succeeded in inducing hyperplasminemia in dogs and rabbits by the intravenous injection of a mixture of human serum and streptokinase.

In their foot-steps, the authors have been engaged from an experimental physiological point of view in the detection and measurement of plasmin substances in the blood, saliva and oral soft tissues in rabbits exposed to various condition.

As described elsewhere, parotid and mixed salivas gave forth the lysis on the standard fibrin plate at streptokinase activation while it failed to appear on the heated plate. Therefore, the conclusion to be drawn is that there do not exist plasmin and activator in the leporine saliva, the existence of proactivator alone being recognized.

When the rabbits were caused to have a higher body temperature as in the experiment two, plasmin activity of both the blood and mixed saliva was known within the thermal range of 41°C to 44°C (rectum temperature).

In the experiments three and four, efforts were made to see whether or not plasmin activity would take place in saliva by causing plasmin activity in the blood by the administration of drugs. Using a different method from that of the authors, OKAMOTO et al. [15] reported on the fact of fibrinolysis by the administration of Mitomycin C. In a 9-day period administration of Mitomycin C in different dosages, the authors found activator in both the blood and saliva on the fifth day. When a larger dosage of Mitomycin C was given in a shorter period of 3 days, activator was found in some of the testing rabbits in common with the blood, parotid and mixed salivas, thus clearly attesting to plasmin activity. When a longer dosage period is compared with a shorter period, there is no appreciable difference in plasmin activity of the blood but, as for plasmin activity of parotid and mixed salivas, the authors can obtain more effective plasmin activity by administering a larger dosage in a comparatively short period of time.

As revealed by the experiment four, when a temporary plasmin activity was induced in the blood the strongest activation took place immediately after the injection of streptokinase, gradually disappearing in proportion to the elapsing of time. Plasmin activity in the blood completely disappeared at the end of 2 hours and plasmin substances were not found either in parotid or mixed salivas. From the fact that when a temporary plasmin activity is induced in the blood this is not accompanied by a similar plasmin activity in the saliva but that when plasmin activity is induced in the blood for a fairly extended period there will be a similar plasmin activity in the saliva, the authors have the impression that the mechanism of plasmin activity in one case differs from that of the other. Latent activation of plasmin substances in man is very strong and it has been suggested by MACFARLANE and BIGGS [4] that if all the latent plasmin substances in the blood were to be activated, fibrinogens of the blood would be destroyed in only a few minutes.

There are many factors responsible for the activation of plasmin substances and when we want to cause plasmin activity experimentally, it is held to be effective to

inflict some surgical invasion on the tissue. According to MACFAELANE [19], however, plasmin activity can be observed in a patient who is brought into the operatory even without a surgical invasion.

By inflicting a surgical invasion on the rabbits by way of extracting the incisors, the authors endorsed the view that a surgical invasion promotes plasmin activity in the blood.

The extraction of plasmin substances in the oral soft tissues in rabbits gave a different pattern from that of man. In the former case, the use of 2 M KSCN solution as an extracting agent failed to bring out plasmin substances, while in man the same extracting agent brought out the fact of plasmin activity in all the cases examined. On the other hand, when 2 M KCl solution was used as an extracting agent tissue activator was attested to only in the gingiva with proactivator being found in the gingiva, palatal and nasal mucosae. From these facts, it can be assumed that, the soft tissues in rabbits being different from those of man, plasmin substances may exist in rabbits to a lesser degree or, contrarily, here more plasmin inhibitor exists.

Conclusions

As the result of their studies dealing with plasmin substances in rabbits, the authors have obtained the following findings.

- 1) The existence of proactivator was recognized in the parotid and mixed salivas.
- 2) When rabbits were artificially caused to have a higher body temperature, plasmin activity took place in the blood and mixed saliva.
- 3) The administration of Mitomycin C induced plasmin activation in the blood, parotid and mixed salivas.
- 4) The administration of streptokinase brought about plasmin activation in the blood but it failed to activate plasmin substances contained in the parotid and mixed salivas.
- 5) Plasmin activity was clearly observed in the blood immediately following the extraction of teeth.
- 6) The extraction by means of 2 M KSCN and 2 M KCl solutions of plasmin substances in the oral soft tissues proved almost of no effect.

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