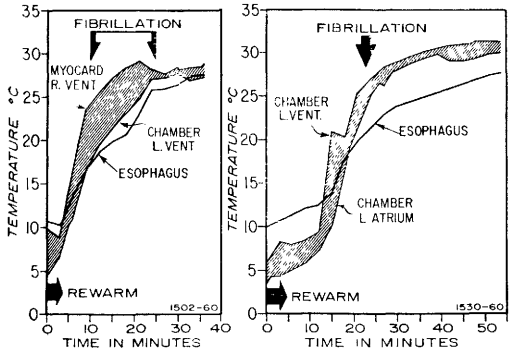

A B

FIG. 2. Cardiac temperature gradients in 2 animals subjected to profound hypothermia and rewarming with fibrillation during rewarming.

dogs from that observed in those which did not fibrillate.

Discussion and conclusion. It is apparent from these studies that induction of hypothermia by extracorporeal perfusion and direct blood cooling results in marked gradients of cardiac temperature. In view of the fact that not a single animal fibrillated during cooling, it would seem that either (1) such gradients *per se* are not a factor in invoking fibrillation, or (2) the gradients must be of greater magnitude than those observed. During rewarming, when fibrillation did occur in 2 instances, temperature patterns were no different from those of animals without arrythmias.

An obvious explanation for the usual temperature gradients encountered and the variation between animals rests on the premise that there is a difference in blood flow through the various portions of the heart. This in turn may be related to catheter position, pump flow rates and other as yet unknown hemodynamic changes. For example, the precise location of the outflow catheters could well determine volume of blood available for circulation through the pulmonary vascular bed. As a consequence of such flow differences, it is also probable that oxygen gradients are likewise present. In view of the possibility that such a situation disposes to fibrillation(4), further study of this facet is contemplated.

Summary. During the production and reversion of profound hypothermia with extracorporeal perfusion temperatures were recorded from various sites throughout the heart. In all animals studied thermal gradients were observed. In some instances during cooling temperature variations as marked as 10°C were recorded. During rewarming similar differences were noted. From these studies it is concluded that thermal gradients alone are not responsible for hypothermic ventricular fibrillation.

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Fibrinolytic Activity in Fluid from Gingival Crevice.* (26308)

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Recent investigations have demonstrated the presence of fibrinolytic systems in various human body fluids(1,2,3). These systems resemble the fibrinolytic system in the blood but differ in that normally no or only very small amounts of plasminogen and inhibitory agents are present(4,5,6,7). Albrechtsen and Thaysen(8) found the fibrinolytic sys-

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tem in parotid and mixed oral saliva to contain small and variable amounts of plasminogen activator and large amounts of a proactivator of plasminogen. Like other body fluids, the saliva contained no plasminogen or inhibitory agents. They thought the plasminogen activator in the parotid saliva might help to resolve any fibrin clots formed in the Ductus Parotideus Stenonii. This investigation appears to be the only one published on the fibrinolytic system of fluids and tissues of the oral cavity.

During the last few years the tissue fluid passage through the gingival crevice has been considered in the etiology of peridontal diseases(9,10).

Discussing the histopathology of peridontal diseases Box(11) stated that the pocket is in reality a capillary space. The intimate relation between crevice and vascular system has been verified by Brill and Krasse(12). Intravenously injected fluorescein sodium was recovered from the crevice of clinically healthy gingivae of dogs in a very short time. The crevice thus seems to take part in the extravascular circulation, and investigation of the plasma constituents of the crevicular fluid appears worth while.

In our investigation we studied fluid from crevices of clinically healthy gingiva for its content of fibrinogen and fibrinolytic factors: plasmin, plasminogen activator, proactivator of plasminogen and plasminogen.

Methods. Teeth and gingivae were carefully dried. The tips of triangles (20 mm^2) of filter paper Munktell nr 3 were then cautiously placed for 3 minutes into the interproximal part of one crevice, care being taken to avoid contamination of the sample with saliva. Pilot studies had shown this time to be sufficient for filter paper to become saturated with tissue fluid. It was possible to repeat this procedure at intervals of 15 minutes without loss of fibrinolytic activity.

Fibrinolytic activity was determined on unheated and heated bovine Astrup fibrin plates as described by Nilsson *et al.*(13). The saturated filter papers were placed immediately on the fibrin plate. The methods described worked with a precision of about ± 15%. Proactivator and plasminogen activity were determined on heated bovine plates after addition of streptokinase or urokinase to the samples and principally as described previously(14).

TABLE I. Fibrinolytic Activity of Tissue Fluid from Gingival Crevices of 11 Females (19-21 Years Old) with Clinically Healthy Gingiva.*

Subjects	Heated plates (mm^2)	Unheated plates (mm^2)
1	104	142
2	105	167
3	52	180
4	50	162
5	62	136
6	88	159
7	120	157
8	47	175
9	95	143
10	76	203
11	134	148

* Activity on the plates was recorded as the diameter product in mm^2 of the lysed zone after 20 hr incubation at 37°C (avg of 3 determinations).

Eluates of the saturated filter papers were tested for fibrinogen as judged by their capacity to react with rabbit antibodies to human fibrinogen (Latex-Anti-Human-Fibrinogen-Reagent, Hyland Labs., Los Angeles).

Results. It is clear from Table I that the tissue fluid possessed plasmin activity. Plasmin activity of the crevicular fluid corresponded approximately to a trypsin solution of 15 μg of trypsin per ml. The lytic area obtained on the unheated plates was considerably larger than that on the heated plates, indicating that the fluid also contained plasminogen activator. After addition of streptokinase or urokinase to the tissue fluid activity on the heated plates increased to approximately the double value indicating presence of plasminogen as well as of proactivator.

After heating at 80°C at pH 2 for 45 minutes the crevicular fluid showed no activity on heated plates while activity on unheated plates remained unchanged. The activator is thus of the thermostabile type.

No plasmin or activator could be demonstrated in the saliva (Gustafsson and Nilsson, to be published) or in samples from other areas of the oral mucosa, not even on the outer surface of the marginal gingiva. Dental plaques on the tooth surface also seemed to

be devoid of plasmin and activator activity.

The activator of the crevicular tissue fluid was inhibited by ε-amino caproic acid and by δ-amino valeric acid which are known to be potent inhibitors of plasminogen activation (15), and not normally to occur in the organism. However, the latter amino acid has been demonstrated in saliva from patients with periodontitis, presumably as a product of local bacterial putrification(16).

When the crevicular fluid was tested against rabbit antibodies to human fibrinogen a positive reaction was obtained indicating presence of the homologous antigen. In the saliva no fibrinogen could be demonstrated by the same test.

It may be objected that the activity on the plates was due not to activator and plasmin but to bacterial proteases. Crude cultures of oral bacteria from the margin of clinically healthy gingiva and from the eroded marginal gingiva of patients with necrotizing gingivitis were added to heated and unheated fibrin plates. Neither of these cultures produced any lysed area. We also collected bacterial samples from the crevice after disinfecting the gingiva with an iodine-glycerine solution(17) before and after insertion of the filter papers. In a few cases culture of samples collected after such disinfection gave no growth indicating absence of viable bacteria. The fibrinolytic activity of such samples did not differ from that of samples giving growth.

Discussion. These last findings and actual existence of the precursor of the fibrinolytic enzyme as well as of the activator in the crevicular tissue fluid indicate that the fibrinolysis noted is attributable to presence of plasmin and activator in the crevicular fluid. The fact that ε-amino caproic acid and δ-amino valeric acid inhibited the activity on the unheated plates may be taken as support for presence of a plasminogen activator and plasminogen. These amino acids do not inhibit the fibrinolytic effect of trypsin and plasmin.

In the discussion on the pathogenesis of peridontal diseases the state of the ground substance has recently been considered, especially the acid mucopolysaccharides and the carbohydrases(18). A major part, however, of the water soluble extractives of connective tissue consists of plasma proteins(19). Our finding of plasmin and plasminogen activator in the crevicular tissue fluid appears also to merit consideration in evaluation of the state and function of the connective tissue of the gingiva. The fibrinolytic factors in the crevicular fluid might be of significance in counteracting the deposition of fibrin and other proteins at the junction between the gingival epithelium and the tooth. Our finding that at least traces of fibrinogen occur even in clinically healthy gingivae argues for a possible formation of fibrin there. In preliminary experiments on patients with necrotizing gingivitis we have found a decrease in fibrinolytic activity in the crevicular fluid at an early stage of the disease, despite the increased flow of tissue fluid in pockets of inflamed gingivae(20).

Summary. The crevicular fluid of 11 female subjects was studied for its content of fibrinolytic factors. It was found to contain plasmin, plasminogen activator, proactivator and plasminogen. The activator activity was inhibited by ε-amino caproic acid and δ-amino valeric acid. Fibrinogen was demonstrated in the crevicular fluid by the reaction of the latter to rabbit antibodies to human fibrinogen. The only one of these factors to be found in the saliva was the proactivator. The significance of an active fibrinolytic system in the crevicular fluid in etiology of peridontal diseases is discussed.

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Supplementary Role of Hydralazine in Reversal of Endotoxin Shock with Metaraminol and Hydrocortisone. (26309)

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Endotoxin shock in the dog can be reversed with a combination of a pressor agent and hydrocortisone, but there still remains the problem of decreased renal function due to reduced renal blood flow(1,2). Because hydralazine has been shown to increase renal blood flow(3-6), the following experiments were designed to measure the additive effect of this agent with metaraminol and hydrocortisone in reversal of canine endotoxin shock.

Methods. Four groups of adult male mongrel dogs, anesthetized with Na Pentobarbital (30 mg/kg), were injected intravenously with a standardized lethal dose of *Escherichia coli* endotoxin, as previously described(1). Measurements of blood pressure and urine output were made continuously for 9 hours, along with hourly determinations of hematocrit and arterial blood pH. Hydralazine,* metaraminol,† and hydrocortisone‡ were administered alone or in combination at that time after endotoxin administration when the animal was manifesting shock with a blood pressure of less than 70 mm Hg systolic, and anuria or oliguria of less than 4 ml/hr.

Group 1 consisted of 12 control dogs given 0.55 mg/kg of endotoxin intravenously. Group 2 involved 6 dogs given the same dose of endotoxin but treated with varying doses of hydralazine according to the output of urine measured at 30 minute intervals. Group 3 included 10 dogs given the lethal dose of endotoxin and then treated with metaraminol and hydrocortisone. Groups of control animals were not treated with either metaraminol or hydrocortisone alone, since previous studies had shown that neither agent reversed endotoxin shock(1). The fourth group of 10 dogs injected with the lethal dose of endotoxin were given hydralazine, and a combination of hydrocortisone and metaraminol in amounts identical to Group 3. That is, one hundred mg of hydrocortisone was infused intravenously, followed by a 1% solution of metaraminol in amounts that maintained systolic blood pressure between 90 and 100 mm of Hg. Hydralazine was administered at varying periods of time after injection of endotoxin.

To clarify the time-dose relationship of hydralazine in the fourth group of animals, it is necessary to review briefly the hemodynamic effects of endotoxin in the dog. Immediately following injection of endotoxin a

* Supplied as hydralazine HCl by Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N. J.

† Supplied as Aramine Bitartrate 10% by Research Division, Merck, Sharp and Dohme, Philadelphia, Pa.

‡ Supplied as Solu-Cortef by Upjohn Co., Kalamazoo, Mich.