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INFLUENCE OF CAPILLARY PERMEABILITY ON FLOW OF TISSUE FLUID INTO GINGIVAL POCKETS

by

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The flow of tissue fluid from subepithelial compartments into clinically healthy gingival pockets has been demonstrated in dogs (*Brill & Krasse,* 1958) and in man (*Brill & Björn,* 1959). The flow of this fluid increases markedly, when gingival structures are stimulated by tooth brushing (*Brill & Krasse,* 1959), chewing (*Brill,* 1959), and in chronic gingivitis (*Brill & Björn,* 1959).

This effect might be due to changes either in the vascular bed, the connective tissue or the epithelial barrier of the gingival pockets, but it is also possible that changes responsible for the effect occur in all three tissues concurrently.

The present study was designed to elucidate the effect of changes in capillary permeability on the flow of tissue fluid into gingival pockets.

The circulatory system of the marginal gingivae comprises a terminal vascular bed including capillaries. (*Parma,* 1939, *Pelzer,* 1940, *Franke,* 1956). The perviousness of capillary walls is generally stated to be variable. (*Krogh,* 1929).

It is well known that histamine increases the permeability of capillaries. A similar effect is observed after mechanical stimulation (*Lewis,* 1927), and in initial stages of inflammation caused by mechanical microtraumata (*Zweifach,* 1953). The result is a copious outflow of fluid and protein material.

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Investigators of capillaries (*Stead & Warren*, 1944 b and *Zweifach*, 1953), however, caution against broad generalisations concerning capillary responses. These may vary under different conditions and in various tissues.

Thus it seemed worth while to assess further the flow of tissue fluid into gingival pockets influenced by histamine, mechanical stimulation and inflammation in gingival structures. This can be done by means of a substance, which does not readily pass through ordinary capillary walls.

A suitable substance is the vital dye T-1824 (mol. w. 960,83) also known as Evans' blue. This dye is very slowly eliminated from the circulation (*Gregersen & Rawson*, 1943). In small concentrations it is bound by the plasma albumin, and in larger concentrations may also be bound by the α-globulin fractions in plasma. (*Rawson*, 1943). Negligible amounts exist as free ions (*Allen & Orahovats*, 1950). Being bound by plasma proteins the dye does not readily pass through "resting" capillary walls. When the permeability of the vessels, however, is increased the dye is carried along with the protein molecules through the vessel walls into extravascular spaces. It can be assumed that if the dye enters subepithelial gingival compartments it will also appear in gingival pockets, because it has been shown by *Brill* (1959) that the fluid entering gingival pockets contains amino acids.

MATERIAL AND METHODS

Two young dogs served as experimental subjects and were studied on two different occasions. On the first occasion, at the outset of the investigation both dogs had healthy mucous membranes including the gingival margins, and at this stage clinically healthy pockets were studied. On the second occasion, inflammation was provoked in the marginal gingivae, and in this inflamed state pockets were again studied. Inflammation was produced in two different ways.

One of the dogs, hereafter called dog I, was for several weeks fed a soft, sticky diet conducive to debris and tartar deposition accompanied by gingivitis. In the other dog (II) rather large biopsy specimens were taken from the gingival margins. This procedure provoked inflammatory reactions. In the second investigational series of dog II pockets were studied, when the V-shaped operated areas were partly filled with granulation tissue covered by new, thin epithelium. Throughout the study dog II was maintained on a hard, gingivitis-preventing diet and did not accumulate food debris or other deposits.

All experiments were carried out while the dogs were anesthetized by intravenous injections of a 5 per cent aqueous solution of "Pentothal"® (Thiopentone Sodium, B.P., or Thiopental-Sodium, U.S.P.). Each experimental session lasted from one to two hours. It was necessary therefore to reinforce the anesthesia during the experiments by additional injections.

About three minutes after the development of anesthesia dog I received an intravenous injection of 12 cc of Evans' blue and dog II received 16 cc. Dog I weighed 29.3 kg and dog II 19.5 kg. Thus dog I weighing more than dog II and receiving the smaller amount of dye, presented a lower concentration of test substance in the blood stream.

Occurrence of dye in the gingival pockets was studied by means of strips of filter paper gently inserted into the pockets. In all instances, the strips were introduced after the injection of the test substance.

The pockets tested belonged to third incisors, canines, premolars and first molars. In all experiments, lingual and vestibular surfaces of the teeth and gums were carefully dried with gauze before samples were taken, and during the experimental procedures the flow of saliva was also checked by means of gauze.

The healthy as well as the inflamed pockets were studied in both dogs in the following manner: when the tongue had turned to a prononunced blue, usually two to three minutes following the injection of the dye, strips of filter paper were inserted and allowed to remain in the pockets for three minutes before removal. Twentyone healthy and sixteen inflamed pockets were tested.

In the experiments concerned with the effect of (1) mechanical stimulation of gingival structures and (2) intravenously injected histamine, only clinically healthy pockets were used.

After injection of the dye, three minutes elapsed before samples from twelve pockets were taken as previously. Next, the same pockets were stimulated by brushing the teeth and gingivae twenty-five times according to the Stillman method (*Stillman*, 1932), and a new series of samples was taken from these pockets. Again the strips remained in the pockets for three minutes.

The effect of histamine was studied in eleven pockets. With one alteration the method just described was also applied in this series. Instead of mechanical stimulation between the collecting of samples, dog I received an intravenous injection of 0.1 mg histamine, and dog II received 1.0 mg.

RESULTS

Intravenously injected Evans' blue was recovered from gingival pockets of resting as well as stimulated structures. The concentrations of the dye on the strips varied with varying conditions of the pockets and varying concentrations of the dye in the blood stream.

Healthy pockets. In dog I which had the lower concentration of dye, the samples were colourless (Fig. 1). A higher concentration as in dog II yielded a bright blue, easily perceptible on the strips (Fig. 2).

Inflamed pockets. In dog I (low concentration and chronic inflammation) the strips were slightly coloured (Fig. 3). In dog II (high concentration and surgically treated) a much heavier blue was seen (Fig. 4). In these series greater amounts of fluid

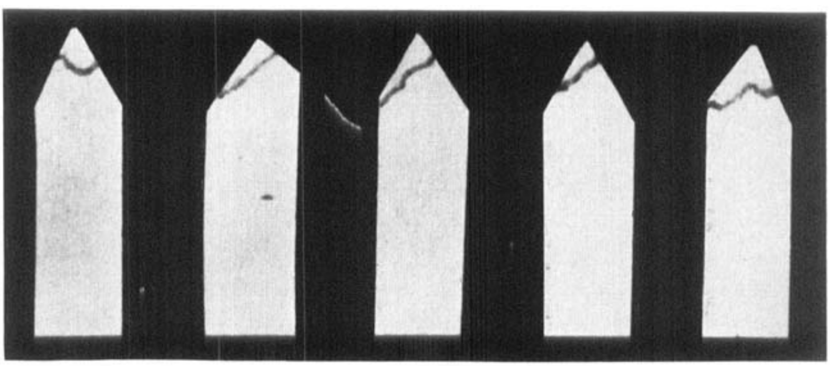

Fig. 1. Colourless samples from healthy pockets in dog I.

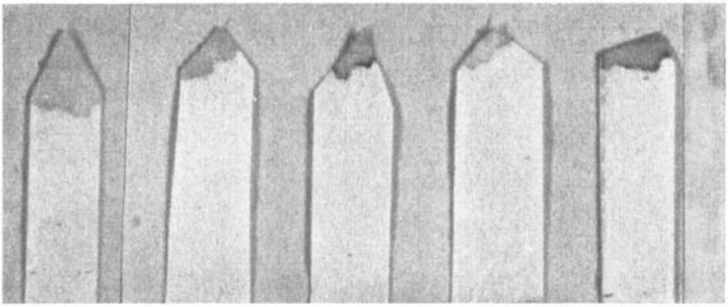

Fig. 2. Stained samples from healthy pockets in dog II.

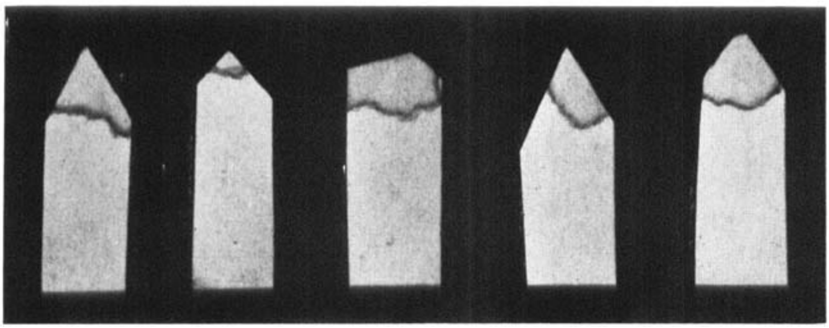

Fig. 3. Stained samples from inflamed pockets in dog I.

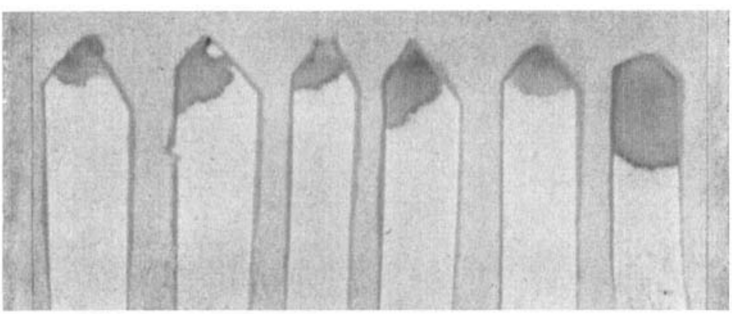

Fig. 4. Stained samples from inflamed pockets in dog II.

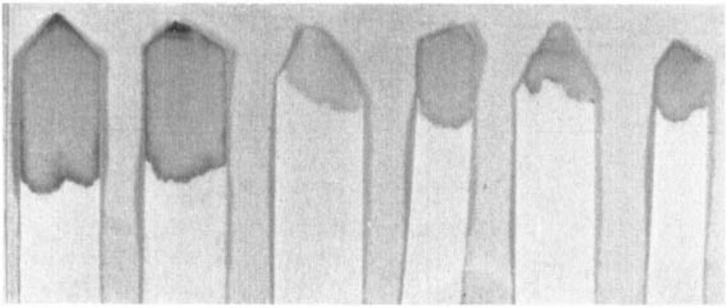

Fig. 5. Stained samples from dog II after mechanical stimulation. The five strips to the left are from the same pockets as those in Fig. 2.

were observed on the strips than in the series from healthy pockets.

Mechanical stimulation. Still greater amounts of stained fluid were recovered from both dogs in these series. Samples from dog I were slightly stained. Samples from dog II were of a heavier shade (Fig. 5).

Influence of histamine. In dog I the injection of 0.1 mg of histamine released a heavy outpour of slightly stained fluid (Fig. 6). The effect, however, was not quite comparable with the effect of mechanical stimulation. In dog II receiving 1.0 mg of histamine the results were questionable in respect of amounts of fluid recovered. In respect of colour, however, a bright blue

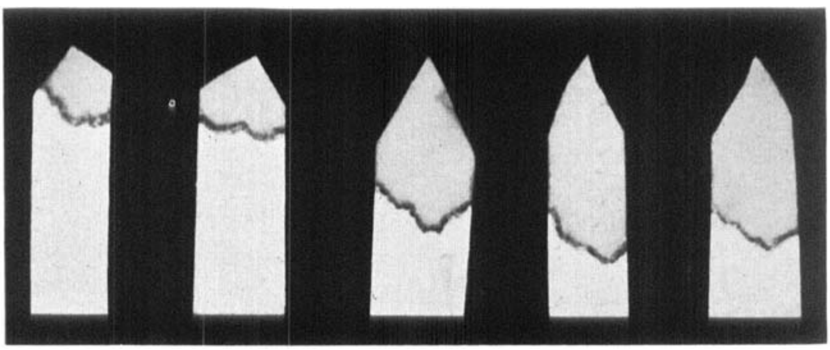

Fig. 6. Stained samples from dog I after histamine injection. Samples from the same pockets as those in Fig. 1.

was clearly visible. The shade was similar to the one observed on strips from healthy pockets in the same dog.

The various light shades and the differences between these shades proved extremely difficult to record photographically.

The results of this study may be summarized and listed in the following order:

(1) Short time mechanical stimulation and surgery recently performed produces a heavy outpour of Evans' blue from vessels into gingival pockets.

(2) Intravenously injected histamine in small concentrations has a similar effect; in large concentrations there is no appreciable effect.

(3) Chronic inflammation stimulates the escape of Evans' blue into gingival pockets appreciably, but compared with (1) the effect is small.

(4) Evans' blue passes into clinically healthy gingival pockets in minimum quantities.

DISCUSSION

Protein-bound Evans' blue from plasma passes ordinary capillary walls in minute amounts and appears in gingival pockets. The concentration of the dye can be increased by (1) mechanical stimulation, (2) intravenously injected histamine and (3) inflammatory reactions. This means that the permeability of capillary walls subjected to these stimuli has increased.

In general the findings are in accord with the views held among pathologists concerning the function of capillary walls. For surveys see *Payling Wright* (1950) and *Florey* (1958).

Ordinarily most protein molecules in the blood are confined within the vessel walls, and only minute quantities are allowed to escape through capillaries (*Zweifach,* 1940). From the present study can be learned that some of the proteinaceous material leaving "resting" vessels, even passes through connective tissue compartments and pocket epithelium, when these tissues are in a clinically healthy state. This observation confirms findings by the author that tissue fluid from unstimulated gingival pockets contains amino acids (*Brill,* 1959). There is, however, an appreciable increase in exudated material in pockets of inflamed gingivae, and a marked increase from surgically treated areas.

When capillary walls are influenced by histamine their permeability is increased (*Lewis,* 1927 and *Stead & Warren,* 1944 a). As pointed out above, however, the reaction may vary in different structures. The results of the present investigation show that intravenously injected histamine in small concentrations has a profound influence on gingival vessels.

Mechanical stimulation of capillaries is also known to enhance the perviousness of the capillary barrier (*Lewis,* 1927, *Zweifach,* 1953). In the present study it was found that the brushing of gingival margins produces an augmented influx into gingival pockets of fluid containing blue stained protein molecules from blood plasma.

The findings made in this study point towards an intimate relationship between the outpour of plasma molecules into gingival pockets and the conditions prevailing in gingival capillary walls.

Bearing in mind that antibodies, natural and specific, are plasma proteins, it is reasonable to expect antibodies to be found among the plasma protein molecules, which have been demonstrated to pass through pocket epithelium into gingival pockets. This being the case the antimicrobial effect in gingival pockets of these substances must be neutralized, before micro-organisms can provoke periodontal disease. A health promoting effect may therefore be ascribed to gingival massage enhancing the occurrence of antimicrobial substances in gingival pockets.

The antimicrobial effect of extravasated fluid oozing through surgically treated areas should also be appreciated. It is not surprising that granulation tissue is perfused with tissue fluid, because the tissue contains numerous newly formed vessels with extremely delicate walls. Granulation tissue may be described as a "physiological" tissue culture in contrast to artificial tissue cultures in laboratories, and it is stated by *Zweifach* (1954) that newly formed vessels in the latter cultures are highly permeable.

SUMMARY

Protein-bound Evans' blue from plasma passes ordinary capillary walls in minute amounts and appears in gingival pockets. The concentration of the dye can be increased by (1) mechanical stimulation, (2) intravenously injected histamine and (3) inflammatory reactions. This means that the permeability of capillary walls subjected to these stimuli has increased.

In the discussion it is pointed out that gingival massage may promote gingival health by increasing the influx of antibodies into gingival pockets.

RESUME

PERMÉABILITÉ CAPILLAIRE ET ÉCOULEMENT DE FLUIDES TISSULAIRES DANS LES CULS-DE-SAC GINGIVAUX

Le liquide contenu dans les culs-de-sac gingivaux renferme d'ordinaire des molécules de protéines provenant du plasma. Ces molécules passent en plus grand nombre des tissus sous-épithéliaux dans les culs-de-sac gingivaux sous l'effet de la stimulation des vaisseaux gingivaux (1) soit par excitation mécanique, (2) soit par injection intraveineuse d'histamine, (3) soit par le fait d'une inflammation chronique. Au cours de la discussion, il est souligné que le massage gingival peut contribuer à la santé gingivale en augmentant le passage d'anticorps dans les culs-de-sac gingivaux.

ZUSAMMENFASSUNG

ÜBER KAPILLARPERMEABILITÄT UND DEN STROM VON GEWEBSFLÜSSIGKEIT IN DIE GINGIVALE TASCHE

Gewöhnlich enthält die Flüssigkeit in der gingivalen Tasche Proteinmoleküle, welche aus dem Plasma stammen. Solche Moleküle kommen in grösserer Menge aus den subepithelialen Geweben in diese Taschen, wenn die Gefässe stimuliert werden entweder (1) mechanisch oder (2) bei intravenös injiziertem Histamin oder (3) bei chronischer Inflammation. Es wird hervorgehoben, dass Massage der Gingiva deren Gesundheit durch das dadurch bedingte Zufliessen von Antikörpern erhöhen könnte.

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