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AZO-DYE-BINDING PROPERTY OF HUMAN GINGIVAL FLUID AT VARIOUS STAGES OF GINGIVITIS

by

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INTRODUCTION

It has been observed that several azo-dyes are bound to various tissue proteins: hemoglobin, globin, albumin and other soluble proteins. This type of chemical reaction between various tissue proteins and diazotized 4-amino-3:1'-dimethyl azobenzene has recently been demonstrated by *Mäkinen and Mäkinen* (1969a). The purpose of this study is to provide a method for applying the above azo-dye-binding property of the gingival fluid to the determination of the degree of inflammation in human gingiva. It is known that the chemical composition of the gingival fluid resembles in many aspects that of other extracellular fluids (*Brill* 1962, *Egelberg* 1963, *Mann* 1963, *Weinstein* and *Mandel* 1964). Passage of this tissue fluid through the crevicular epithelium is increased by mechanical stimulation of the teeth and gingiva (*Brill and Krasse* 1959). The flow of gingival fluid is also markedly increased in gingivitis (*Brill and Björn* 1959).

MATERIALS AND METHODS

The material was collected from 46 individuals, using filter paper strips (2×10 mm, Whatman No. 1) according to *Löe and Holm-Pedersen* (1965).

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The teeth and the surrounding tissue were thoroughly dried by a blast of air and kept dry with cotton rolls in the vestibule. The strips were inserted carefully in the entrance of the crevices (2 strips per individual, in different crevices). The sampling period was 3 min. The gingiva was scored according to the gingival index (GI) suggested by *Löe* and *Silness* (1963). The clinical assesment was always performed prior to the collecting of fluid.

The paper strips were stored in a freeze-box (-20°) until the analyses were carried out. The strips were immersed in 0.4 ml of 0.025 M phosphate buffer, pH 7.0, at room temperature ($+26^{\circ}$). The tubes were gently shaken for 10 min. 0.1 ml of the resulting solution was pipetted to 6 ml test tubes containing 0.3 ml of the above buffer, 0.1 ml of freshly prepared 0.85×10^{-3} M aqueous solution of diazotized 4-amino-3:1'-dimethyl azobenzene HCl (Fast Garnet Salt GBC; G. T. Gurr, Ltd., London, England) and 0.1 ml of water. The tubes were kept for 30 min. at $+37^{\circ}$, after which 0.2 ml of 1 mM 2-naphthylamine solution was added. The 2-naphthylamine solution was made after first dissolving 144 mg in 10 ml ethanol in a 100 ml volumetric flask. The volume was made to 100 ml with 1.0 M acetic buffer, pH 4.2, containing 10 % (v/v) tween-20 (polyoxyalkylene sorbitan mono-laurate). After standing 30 min. at room temperature the extinction of red colour was measured at $525 \text{ m}\mu$ on a Hitachi Perkin Elmer Model 139 spectrophotometer against water. Simultaneously reaction mixtures were prepared where either the diazonium compound or the gingival fluid preparation was omitted. These blank mixtures were treated as above. Blank experiments were run concurrently to reveal the contribution of the reagents and the coloured protein preparations to the extinction, and the appropriate correction was applied. Standard curves were prepared of different diazonium salt concentrations.

RESULTS

A typical result from one set of measurements is given in Table I. The ability of the tissue fluid samples to accomplish azo-dye-binding in the reaction mixtures is shown by the lower extinction obtained for the test mixtures, the unreacted azo-dye displaying an extinction of 0.207 and the protein blank an extinction 0.010. It is seen that all samples caused some binding of the azo-dye. The Table shows, however, that samples obtained from patients suffering from moderate or severe gingivitis caused clearly lower extinction or more binding to the azo-dye. This lowering of the extinction, or the increased ability of the tissue fluid samples to bind the diazonium compound, was applied in chemically determining the degree of inflamma-

Table I.

An example from a typical experiment to reveal the degree of inflammation of the gingival pocket by the azo-dye-binding method. The extinctions given are average figures from two measurements. The extinction given by the unbound azo-dye in this experiment was 0.207

Patient No	Extinction of the reaction mixture	Extinction of the blank	ΔE	Gingival index (GI)
34	0.186	0.010	0.176	1
35	0.185	0.012	0.173	2
36	0.182	0.015	0.168	2
37	0.186	0.016	0.170	2
38	0.158	0.022	0.136	2
39	0.188	0.010	0.178	2
40	0.179	0.012	0.167	2
41	0.192	0.012	0.180	1
42	0.173	0.012	0.161	2
43	0.176	0.012	0.164	1
44	0.197	0.010	0.187	1
45	0.197	0.010	0.187	1
46	0.188	0.008	0.180	1
47	0.200	0.008	0.192	1

tion of the gingiva. All the data compiled in this study is presented in Figs. 1 and 2. The results are similar to those in Table I, i.e. the amount of the azo-dye-binding components in the gingival fluid was noticeably higher in samples obtained from patients with gingivitis scored to GI 2 and GI 3 than from samples representing slight gingivitis (GI = 1). These chemically determined results were always consistent with those obtained by clinical diagnosis.

Emphasis is drawn to one important criterion for selecting the material in this investigation: nonacceptance of samples displaying red colour from contamination by blood. For patients scored to group GI 3 the gingival fluid samples had readily become red-coloured from this leakage of blood. As a result, data is offered for only one case scored to GI 3.

DISCUSSION

The azo-dye-binding property of various soluble proteins here used to reveal the degree of human gingiva inflammation is not dependent on the reaction of the dye to any specific protein or other tissue components, although some proteins may react more rapidly than others. It is evident

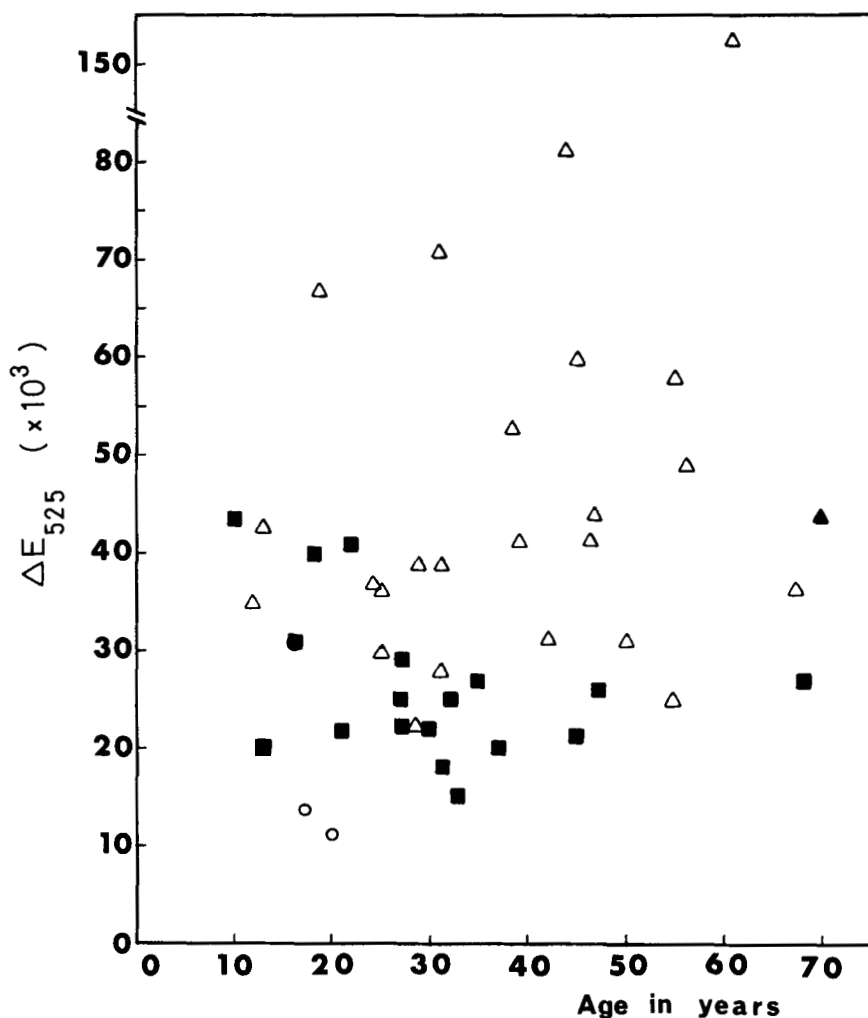


Fig. 1. The azo-dye-binding capacity of gingival fluid in various stages of inflammation.

- ▲ = gingival index (GI) 3
- △ = " " 2
- = " " 1
- = " " 0 (inflammation was not clinically observed)

The figures of the ordinate were obtained by the following calculation: azo-dye-binding capacity: $[E_{\text{unbound azo-dye}} - (E_{\text{reaction mixture}} + E_{\text{protein}})] \times 10^3$ where E refers to E₅₂₅ measured for the azo-dye.

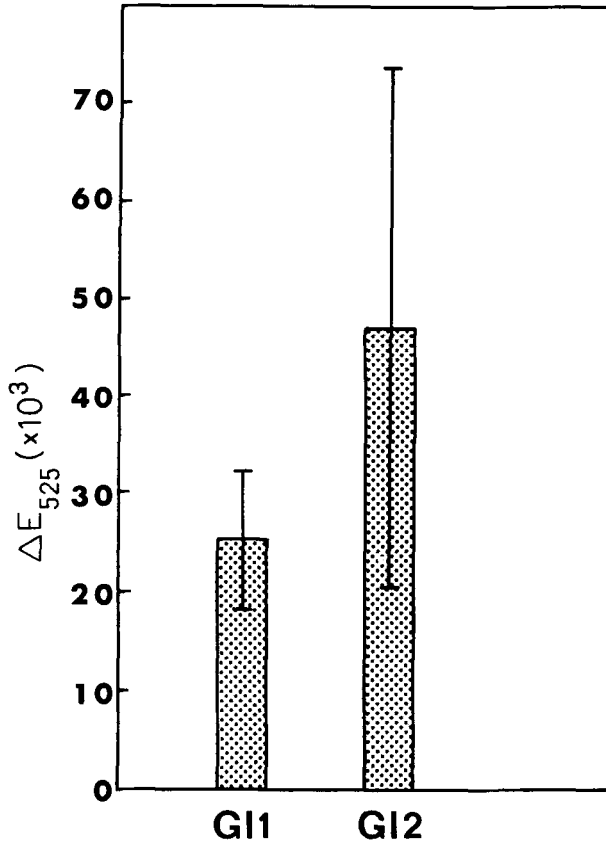


Fig. 2. The values for samples representing GI 1 were significantly lower than that for samples representing GI 2 ($t = 2.77$, $P < 0.01$).

that a large number of various azo-dyes are similarly bound by various proteins. Observations made of the chemical basis of azo-dye-binding is presented elsewhere, along with a short review of the literature (Mäkinen & Mäkinen 1969b). It bears mention that the azo-dye used in this study is bound particularly to hemoglobin, globin, and related proteins and serum albumin. Other experiments, not presented here in detail, have shown that bacterial protein preparations also contain components capable of reacting to the azo-dye.

The most important finding is the correlation between the ability of gingival fluid to bind the diazonium compound and a clinically determined

diagnosis of gingiva. It is not even necessary to know what kind of proteins or other components of the gingival fluid react to the azo-dye. It may also be that mere extinctions can be employed as a type of gingival index to describe the degree of inflammation.

SUMMARY

The purpose of this study was to provide a method for applying the azo-dye-binding property of gingival fluid to the determination of the degree of inflammation in human gingiva. The material was collected with filter paper strips from 46 individuals.

The results revealed that the values for samples representing gingival index (GI) 1 were significantly lower than those for samples representing GI 2.

It is mentioned that the azo-dye used in this study is bound particularly to hemoglobin, globin and related proteins and serum albumin, though it is not at all necessary to know what kind of protein or other components of the gingival fluid react to the azo-dye. The most important finding is the correlation between the dye-binding, ability of gingival fluid and the clinically determined diagnosis of gingiva.

RÉSUMÉ

APTITUDE DU FLUIDE GINGIVAL HUMAIN À FIXER LES COLORANTS AZOÏQUES AU COURS DES GINGIVITES DE DIFFÉRENTS DEGRÉS

Cette étude a eu pour but l'élaboration d'une technique utilisant pour la détermination du degré d'inflammation de la gencive humaine l'aptitude du fluide gingival à fixer les colorants azoïques. Le matériel, provenant de 46 sujets, a été recueilli au moyen de bandes de papier filtre.

Il ressort des résultats que les valeurs obtenues pour les échantillons correspondant à l'indice gingival (IG) 1 étaient significativement moins élevées que les valeurs correspondant à IG 2.

L'auteur indique que le colorant azoïque utilisé dans cette étude se fixe particulièrement sur l'hémoglobine, la globine et les protéines de ce genre et sur l'albumine sérique, mais qu'il n'est pas du tout nécessaire de savoir quelle sorte de protéine ou d'autres éléments du fluide gingival réagissent au colorant azoïque. Le fait le plus important ressortant de cette étude est la corrélation trouvée entre l'aptitude du fluide gingival à fixer le colorant et le diagnostic établi par examen clinique de la gencive.

ZUSAMMENFASSUNG

FÄHIGKEIT DER MENSCHLICHEN GINGIVATASCHEFLÜSSIGKEIT ZUR BINDUNG VON ATSOFFARBE IN ZAHNFLEISCHENTZÜNDUNGEN VERSCHIEDENER SCHWEREGRADE

Diese Untersuchung erzielte das Herausfinden einer Methode zur Bestimmung des Schweregrades menschlicher Zahnfleischentzündungen durch Anwendung der Fähigkeit der Zahnfleischtaschefflüssigkeit zur Bindung von Atsoffarbe.

Das Material wurde bei 46 Individuen mit Löschpapierstreifen entnommen.

Die Resultate zeigten, dass die Proben, die den Gingivalindex (GI) 1 vertreten niedrigere Werte gaben, als die den GI 2 vertretenden. Es ist bewiesen, dass die in dieser Arbeit angewandte Atsoffarbe sich teils mit Hämoglobin, Globin und dergleichen Proteinen und Serumalbumin verbindet. Es ist doch nicht wichtig, Auskunft darüber einzuholen, welche Proteine oder andere Komponente der Zahnfleischtaschefflüssigkeit mit der Atsoffarbe reagieren. Das Wichtigste besteht darin, dass die Eigenschaft der Gingivataschefflüssigkeit mit der klinischen Diagnose im Einklang steht.

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