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THE RELATIONSHIP BETWEEN THE SERUM PROTEIN PATTERN AND THE NUMBER OF LACTOBACILLI IN THE SALIVA

by

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INTRODUCTION

The occurrence and the ecology of lactobacilli in the saliva have been made the subject of numerous investigations. Yet it has not been possible definitely to clear up the relation of these bacteria to caries or the prerequisites for their growth in the oral cavity. *Green & Dodd* (1956), for example, found the oral flora and the number of lactobacilli therein to be fairly constant irrespective of the degree of resistance or susceptibility to caries and ascribed this finding to hereditary factors. *Goodman et al.* (1959) found this to hold true for streptococci and some other bacteria, but not for lactobacilli. It may, however, now be regarded as established that high caries activity is often associated with a high lactobacillus count, but this rule is by no means without exceptions. Thus, *Krasse* (1954) found that the lactobacillus test in the saliva was sometimes even negative in persons with high caries activity and that persons resistant to caries sometimes had a high lactobacillus count.

It is widely believed that lactobacilli thrive best at low pH, which is to a large extent brought about and maintained by the lactobacilli themselves and that this pH offers protection to the individual's adapted flora against invasion and establishment of pathogenic bacteria (*Lammers* 1953, *Stainer et al.* 1958, and

others). This means that the immunological response demonstrable in the blood serum on antibody titration (*Nordh* 1960), for example, will vary to some extent with the lactobacillus count. In this connection it might be mentioned that *Nordh* (1963) demonstrated a certain correlation between the serum protein pattern and the lactobacillus count. Since, however, the material used in that investigation included persons with roentgenological evidence suggesting the presence of chronic oral inflammatory conditions, it was not possible to decide to what extent the electrophoretic changes should be ascribed to the lactobacillus count or the inflammatory conditions.

It was therefore thought desirable to study the electrophoretic patterns in two extreme groups, one with a very low, the other with a very high, lactobacillus count but on the whole without chronic oral inflammatory conditions.

MATERIAL AND METHODS

The material consisted of employees of the Royal Dental School, Malmö, and of patients in whom the number of lactobacilli in the saliva had been assessed on at least two, and frequently more, occasions at the Department of Bacteriology of the school.

Group A. This group was made up of persons in whom the lactobacillus tests were on most occasions negative. The group consisted of 36 individuals (21 men and 15 women). The lactobacillus test was negative on two occasions or more in 24 individuals. The lactobacillus test showed less than 10,000 lactobacilli/ml on one occasion but was otherwise negative in 6 individuals and in 6 others the test showed a count of at most 10,000/ml but without a negative test. In this group the prevalence of caries was very low and none of the subjects had active caries at the time of the examination. Neither were any radiolucent areas seen around the teeth.

Group B. This group was made up of 47 individuals (26 men and 21 women) in whom a lactobacillus count of more than 1,000,000/ml had been found on at least two, and often on more occasions. All of them had received thorough dental treatment before the tests.

No persons with roentgenologically demonstrable evidence of chronic oral inflammatory conditions were accepted.

The normal values of the medical laboratory were based on examinations of apparently healthy persons. This *control series* was largely comparable to groups A and B in respect of the mean and range of variation of age.

Samples of the blood and of the saliva were collected simultaneously before breakfast. The total protein was determined by a modified Biuret technique (*Reinhold 1953*). Paper electrophoresis was done by the method of *Laurell et al. (1956)*. The material was examined at the medical and bacteriological laboratories of the Royal Dental School, Malmö.

RESULTS AND DISCUSSION

The results of the electrophoretic analysis are given in Table 1.

Table 1

Mean and standard error of the mean of the electrophoretic fractions in the material

Group	g protein per 100 ml serum M \pm ϵ (M)	Total protein	Albumin	α_1	α_2	β_1	β_2	γ
A								
Lactobacill-0	M	7.52	5.356	0.270	0.437	0.436	0.234	0.708
n = 36	ϵ (M)	0.061	0.065	0.005	0.012	0.008	0.007	0.026
B								
Lactobacill-mill.	M	7.58	5.035	0.311	0.537	0.521	0.250	0.892
n = 47	ϵ (M)	0.060	0.065	0.007	0.019	0.006	0.007	0.026
Normal values of the laboratory	M	7.46	5.08	0.29	0.47	0.51	0.21	0.89
n = 268	ϵ (M)	0.032	0.024	0.002	0.004	0.004	0.002	0.013

It is clear from Table 1 that, broadly speaking, the means found for group A tended to be above; those for group B, below, the normal laboratory means. Also the mean bacillus count in the normal group probably lay between those in groups A and B and closer to the latter.

In group B the albumin was significantly ** lower, and the

α_1 - and α_2 -fractions significantly *** higher, than the corresponding values in group A. This implies the presence, in group B, of a reaction in the serum to environmental primary inflammatory cellular damage (*Odenthal* 1958) with tissue destruction and release of decomposition products. Compared with group A, the normals also showed evidence of an inflammatory reaction, though slight.

Assuming that the values found for albumin α_1 and α_2 in group B reflected an acute infection (see *Nordh* 1964) the mean β_1 -fraction should also have been lower in this group than in group A. It was not, however. On the contrary, it was significantly *** higher than in group A.

One might imagine this unexpected result to be due to other components, and then probably to a difference in the amount of lipoproteins. Only a smaller part of the β -lipoproteins migrates in the β_1 -globulin fraction, but an increase in the amount of β -lipoproteins in group B could not be excluded. In an attempt to reveal any difference in amount of β -lipoprotein between the two groups the relationship between the quantities of these fractions was estimated by determination of the nephelometric index after precipitation with heparin (*Burstein & Samaille* 1956, *Hitzig* 1963). The results are seen in Table 2.

Table 2
Nephelometric index in groups A and B after precipitation of lipoproteins with heparin

Group	M	$\pm s$ (M)
A	54.9	\pm 2.2
B	57.2	\pm 1.8

The nephelometric index is directly proportional to the amount of β -lipoproteins. The difference found between the two groups has no degree of significance regarding the concentration of β -lipoproteins and could thus not be held responsible for the difference in the globulin value of the β_1 -fractions. Owing to the existence of inflammatory conditions suggested by the serum protein pattern in group B, one might have expected an increase of the immunoglobulins too. As a matter of fact the mean γ -globulin value was found to be significantly ** higher for group B.

About 70 per cent of the γ -globulins consist of immunoglobulins (Laurell 1963). However, the difference seems to be too small for making it probable that the relatively small amount of immunoglobulins which migrate in the β_1 -fraction ($=\gamma_{1A}$) should be able to produce the marked difference in this fraction between groups A and B. The slight difference between the mean value for group B and the normal laboratory mean did not suggest any substantial increase of the immunoglobulins in group B either.

One must therefore suspect that the difference was due mainly to a difference in the amount of transferrin, the iron binding serum globulin which is the bulk of the β_1 -fraction, between the two groups. This is also apparent from a comparison of the amount of albumin in the two groups and in the normal group. A low albumin value is usually accompanied by a low β_1 -globulin value due to a low transferrin value. The relatively low albumin value in group B, however, was accompanied in this case by a high β_1 -globulin value, and since this can hardly be ascribed to a high lipoprotein content, there was probably a difference in the amount of transferrin.

There existed no hereditary differences in type of transferrin with a different rate of migration between the two groups. On starch gel electrophoresis all of the individuals in both groups were found to belong to type TfCC (Smithies 1955, Smithies & Walker 1956, Hitzig 1963).

The difference between groups A and B regarding the β_2 -fraction was not significant. This fraction, however, was difficult to evaluate because the sera had been stored before analysis, kept at -15°C . During storage of the serum, part of the β_{1C} -immunoglobulin may convert to β_{1A} which migrate with the β_1 -fraction.

CONCLUSION

It may be concluded that compared to persons with a negative lactobacillus count (group A), individuals with a lactobacillus count of more than 1,000,000/ml saliva (group B) showed an inflammatory type of serum protein pattern. This reaction was noted both for environmental primary cellular damage and sec-

ondary response of the lymphoid tissue. The transferrin level also may be assumed to be lower in the group without lactobacilli in saliva. This might in turn indicate either a greater iron binding capacity in group A or a larger requirement of iron in group B (*Brendstrup* 1953, *Laurell* 1960, 1963).

SUMMARY

The purpose of the present investigation was to compare the electrophoretic patterns of two groups of individuals with different numbers of lactobacilli in their saliva. In one group (A) the lactobacillus test was negative on various occasions; in the other (B), more than 1,000,000/ml saliva. In the former group the frequency of caries was very low; none had active caries and no radiolucent areas were seen around the teeth. In the latter group which included no persons with radiolucent periradicular areas, the frequency of caries was high. The normal laboratory electrophoretic values were based on examination of healthy persons in other connections than dental.

Compared to the group with negative lactobacillus tests, the group with a high lactobacillus count showed a protein pattern typical of inflammation. The reaction pattern could be interpreted as a response to an environmental primary inflammatory cellular injury with a low albumin value, increased α -globulins and as a secondary response of the lymphoid tissue, in form of increased immunoglobulin values. In addition, owing to the strongly significant difference between the groups regarding the β_1 -globulin fraction, there was reason to assume that the group with negative lactobacillus tests had a lower concentration of transferrin, the iron binding serum globulin. This might in turn indicate that the individuals with a negative lactobacillus test had a greater iron binding capacity or that the individuals with high lactobacillus counts had a increased need of iron.

RÉSUMÉ

RELATION ENTRE LES RÉSULTATS DE L'ÉTUDE DES PROTÉINES SÉRIQUES ET LE NOMBRE DE LACTOBACILLES DANS LA SALIVE

Le but de la présente étude a été de comparer les résultats des examens électrophorétiques chez deux groupes de sujets ayant

différents taux de lactobacilles dans la salive. Dans l'un des groupes, le test de recherche des lactobacilles était négatif à plusieurs reprises; dans l'autre groupe, le taux était de plus de 1.000.000/ml de salive. Dans le premier groupe, la fréquence de la carie était très basse; aucun de ces sujets ne présentaient de carie en activité, et aucune zone radioclaire n'était visible autour des dents. Dans le second groupe, qui ne comprenait aucun sujet présentant des zones radioclares périradiculaires, la fréquence de la carie était élevée. Les valeurs électrophorétiques normales de laboratoire étaient basées sur l'examen de personnes en bonne santé, en dehors du point de vue odontologique.

Par comparaison avec le groupe présentant des tests de recherche des lactobacilles négatifs, les résultats de l'étude des protéines du groupe ayant un taux de lactobacilles élevé étaient caractéristiques de l'inflammation. Le mode de réaction pouvait être interprété comme étant une réponse à une lésion cellulaire inflammatoire primaire de voisinage, avec une faible valeur d'albumines, augmentation des α -globulines et comme une réponse secondaire du tissu lymphoïde, sous forme d'une augmentation de la valeur des immuno-globulines. De plus, en raison de la différence fortement significative existant entre les groupes en ce qui concerne la fraction des β_1 -globulines, il était raisonnable de supposer que le groupe présentant des tests de recherche des lactobacilles négatifs avait une concentration moins élevée de transferrine, la sérum-globuline qui fixe le fer. Ceci peut à son tour indiquer que les sujets ayant un test de recherche des lactobacilles négatif présenteraient une plus grande aptitude à fixer le fer, ou que les sujets ayant un taux de lactobacilles élevé présenteraient une augmentation des besoins en fer.

ZUSAMMENFASSUNG

VERHÄLTNIS ZWISCHEN SERUMPROTEINSPEKTRUM UND LACTOBACILLENZAHLEN DES SPEICHEL

Der Zweck der vorliegenden Arbeit war, die Elektroferogramme bei zwei Gruppen von Personen mit verschiedenen Lactobacillenzahlen in dem Speichel zu studieren. Die eine Gruppe hatte bei mehreren Untersuchungsgelegenheiten negative Lactobacillenzahlen gezeigt, die andere Gruppe mehr als eine Million Lacto-

bazillen pro ml Speichel. In der ersteren Gruppe war die Kariesfrequenz sehr niedrig; keine akute Karies und keine radioluzente Gebiete um die Zähne herum lagen vor. In der letzteren Gruppe war die Kariesfrequenz hoch, jeder Fall mit radioluzenten, periodikulären Gebieten war aber ausgeschlossen worden. Die elektrophoretischen Normalwerte des Laboratoriums waren auf Seren gesunder Personen gegründet, die für andere Zwecke untersucht worden waren.

Dem Ergebnis ging hervor, dass die Gruppe mit hohen Lactobazillenzahlen im Speichel ein typisches entzündliches Serumproteinspektrum aufwies im Vergleich mit der lactobazillennegativen Gruppe. Das Reaktionsspektrum könnte sowohl als Antwort eines milieubedingten, primär entzündlichen Zellschadens mit erniedertem Albuminwert und verhöhtem α -Globulinwert, als auch eine sekundäre Antwort lymphoider Gewebe in Gestalt verhöhter Immunglobulinwerte erklärt werden. Ausserdem scheint man infolge eines stark signifikanten Unterschiedes zwischen den β_1 -Fraktionen der Gruppen annehmen zu können, dass die Gruppe mit negativem Lactobazillentest eine quantitativ niedrigere Menge Transferrin, das eisenbindende Serumglobulin, hatte. Dieses könnte darauf hindeuten, dass entweder die lactobazillennegativen Personen grössere eisenbindende Fähigkeit hatten, oder auch dass Personen mit höheren Lactobazillenzahlen einen erhöhten Eisenbedarf hatten.

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