

PENETRATION OF HUMAN ALBUMIN THROUGH THE ORAL MUCOSA OF GUINEA-PIGS IMMUNIZED TO THIS PROTEIN

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Summary—Penetration of labelled human albumin through the oral mucosa of guinea-pigs was estimated by scintillation counting of serum samples. Higher serum radioactivity was observed in animals immunized to the albumin than in non-immunized animals. Gel-chromatography of the serum revealed a substantial degradation of the albumin, but immune precipitation confirmed penetration of antigenically intact albumin. The results suggest the possibility of an increase in permeability of the mucosal basement membrane, by deposition of immune complexes.

INTRODUCTION

Alpha-amylase (Wieriks, 1964), streptokinase (Bachman, 1968), horseradish peroxidase (McDougall, 1970, 1971, 1972) and albumin (Tolo, 1971) are among proteins reported to penetrate the oral mucosa. The presence of antibodies may interfere with the penetration of antigens through formation of immune complexes. It is of interest that immunoglobulins have been demonstrated in the intercellular spaces of human gingival, buccal and pharyngeal epithelium (Brandtzæg and Kraus, 1965; Brandtzæg, 1966; Genco and Krygier, 1972). Immune complexes may also activate the complement system, and the filtering structures of the oral mucosa may become targets for enzymes released from neutrophil leukocytes.

The purpose of the present study was to examine the penetration of human albumin through the oral mucosa of guinea-pigs immunized to this protein.

MATERIAL AND METHODS

Animals

Seven guinea-pigs weighing approximately 500 g were used. Four animals were immunized by three subcutaneous injections of 0.5 mg human albumin (Cohn Fr. V, Hyland Laboratories, US), emulsified with 0.5 ml Freund's complete adjuvant (Difco, US), over a period of 10 days. After 4 weeks, a booster dose of 1 mg of albumin was given without adjuvant. Two weeks later sera from all the immunized animals showed strong precipitation lines against human albumin (1 mg/ml) by double diffusion in agar gel. Sera from the three non-immunized animals showed no precipitation lines.

Tracer molecules

Human albumin was tritium-labelled by exposure to tritium gas (Wilzbach, 1957) at the Radiochemical

Centre, Amersham, England. The labelled protein was purified by repeated gel filtration on a column, size 1.5×30 cm, packed with Bio-Gel P 150 which had been equilibrated with a 0.1 M aqueous solution of NaCl buffered at pH 7.3 with 0.05 M tris/HCl. This solution was also used for elution. The purified protein was eluted in a single peak (K_{av} 0.33) and was stored in a concentration of 0.57 mg/ml at 6°C. The K_{av} of a solute in gel filtration is the partition coefficient between the liquid phase and the gel phase.

Penetration experiments

The animals received an intramuscular injection of fluanison + phenantyl citrate (0.8 ml Hypnorm, Mekos) and the oesophagus was ligated just beneath the pharynx. Two millilitres of blood was collected by heart puncture (T_0 sample). One millilitre of the test solution (0.57 mg/ml albumin) was applied to the sublingual and gingival mucosa for 90 min. Immediately afterwards, another sample of blood (T_{90} sample) was obtained by heart puncture. The animals were killed by an intraperitoneal injection of Nembutal and biopsies of the tongue, gingiva, submandibular lymph nodes, lung and liver were rapidly placed in Lawdovsky's fixative for preparation of autoradiographs as described by Tolo (1971).

Scintillation counting

Serum was obtained within 15 min after coagulation at room temperature. The blood samples were centrifuged at 1500 *g* for 10 min and the serum was stored at 6°C. Aliquotes of 0.2 ml serum were mixed in a counting vial with 1.5 ml Soluene (Packard Instr. Co., US)/isopropanol (1:1) and 0.5 ml of a 30 per cent solution of hydrogen peroxide.

After 1 hr at room temperature, 15 ml Instagel (Packard Instr. Co., US) containing 0.3 ml acetic acid per 100 ml was added. The samples were stored in darkness at 6°C for 24 hr before being counted in a

Packard Tri-Carb scintillation counter. Fractions obtained after chromatography of serum on agarose were counted after addition of 10 ml Instagel with acetic acid to 1 ml fractions.

Gel-chromatography

T_{90} serum from immunized animals was chromatographed at 6°C on a column, size 1.5 × 90 cm, of Bio-Gel A 1.5, mesh 100–200, equilibrated with 0.05 M tris/HCl buffer, pH 7.3, containing 0.1 M NaCl and 0.02 per cent sodium azide. The flow rate (descending) was 8 ml/cm²/hr. The void volume (V_0) was determined to 50 ml by Blue Dextran 2000 (Pharmacia, Uppsala, Sweden), and the total volume (V_t) was 154 ml. Fractions of 1 ml were collected by a LKB 7000 Ultrarac collector (LKB Laboratories, Stockholm, Sweden) equipped with a drop counter. Radioactivity (counts/min) and optical density (OD₂₈₀) at 280 nm/cm were recorded in all fractions.

Immune precipitation

In order to test whether the radioactive material in T_{90} serum from immunized animals could be precipitated through addition of human albumin, 300 µl of serially diluted non-labelled human albumin was added to 300 µl of serum. The samples were incubated at 37°C for 1 hr, stored at 6°C for 12 hr and centrifuged at 10,000 *g* for 1 hr at 0°C and the radioactivity was counted in the supernatant. Addition of 0.66 mg human albumin to 1 ml T_{90} serum gave optimal precipitation, and this concentration was used for precipitation of all T_{90} samples from the immunized animals.

T_{90} serum from non-immunized animals was precipitated with rabbit antiserum to human albumin (Cat. 10001, Dakopatts, Copenhagen, Denmark). Incubation of 300 µl serum with 25 µl antiserum gave optimal precipitation, and was used for precipitation of all T_{90} serum samples from non-immunized animals.

In a control for non-specific precipitation of the radioactive material in T_{90} serum from immunized ani-

mals, an amount of ¹²⁵I-labelled human IgA (a gift from Dr. Per Brandtzæg, Rikshospitalet, Oslo), corresponding to 310 counts/min/ml, was added to the T_{90} serum before this was incubated with albumin. After precipitation, the total ¹²⁵I-activity of the serum sample and only one-third of the ³H-activity was recovered in the supernatant.

Table 1. Radioactivity of serum expressed in counts/min before and after 90 min exposure of the oral mucosa of guinea-pigs to tritiated human albumin. The values include a background of 16.7 counts/min, and the counting error at this level was calculated to 1.3 counts/min

Animals	T_0 serum	T_{90} serum
Non-immunized		
2A	15.2	27.9
4A	16.8	28.0
6A	18.1	28.0
Immunized		
1A	16.1	58.3
3A	15.9	30.6
5A	20.7	42.7
7A	14.0	41.5

RESULTS

Penetration experiments

Background values of radioactivity were found in all T_0 samples (Table 1). In non-immunized animals, the T_0 values of serum radioactivity were consistently around 28 counts/min. The immunized animals showed higher radioactivity in the T_{90} serum. The wide range of T_{90} values in this group may reflect a corresponding range in antibody titres.

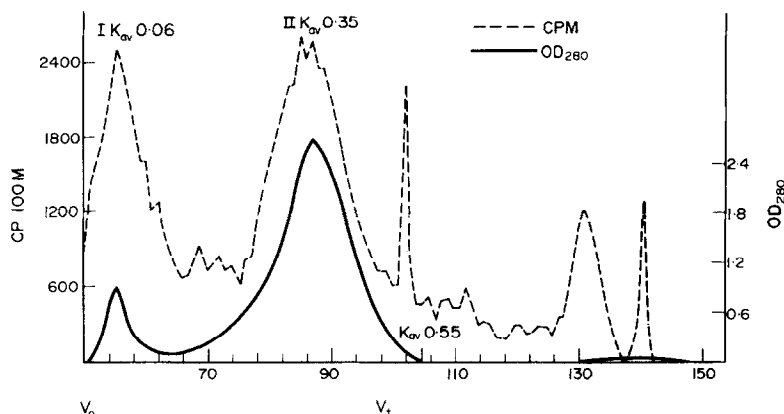


Fig. 1. OD_{280 nm} and scintillation profile of serum from guinea-pigs which had the oral mucosa exposed to tritiated albumin for 90 min. The serum was chromatographed on a column 1.5 × 90 cm packed with Bio-Gel A 1.5 agarose, equilibrated with 0.05 M tris/HCl buffer pH 7.3, containing 0.1 M NaCl. The same buffer was used for elution.

Gel-chromatography

The proteins in T_{90} serum from immunized animals were eluted in two peaks (Fig. 1). Peak I had a K_{av} of 0.06 and peak II had a K_{av} of 0.35. The ratio (counts/min/OD₂₈₀) of peak I was seven times higher than that of peak II. Peak I accounted for 28 per cent of the total radioactivity of the sample, peak II accounted for 52 per cent, and the remaining 20 per cent of the radioactivity was eluted with material having a K_{av} of more than 0.55.

Immune precipitation

After precipitation of T_{90} serum from immunized animals with human albumin, about 28 per cent of the total activity of the samples was recovered in the supernatants (Table 2). Precipitation of T_{90} serum from non-immunized animals with rabbit antiserum to human albumin similarly reduced the radioactivity which could be recovered in the supernatant to 20 per cent of the total activity of the sample.

Table 2. Radioactivity of T_{90} serum (counts/min) before and after precipitation with antialbumin (non-immunized animals) or human albumin (immunized animals). The values include a background of 16.7 counts/min

Animals	Serum before precip.	Serum after precip.	Net reduction (%)
Non-immunized			
2A	27.9	18.8	81
4A	28.0	21.2	60
6A	28	15.6	100
Immunized			
1A	58.3	28.6	71
3A	30.6	22.3	62
5A	42.7	22.2	79
7A	41.5	20.4	85

Autoradiography

Radioactive material was observed in non-keratinized parts of the oral mucosa and in all other tissues examined. No accumulation of radioactive material was seen superficial to the basement membrane in biopsies from the oral mucosa.

DISCUSSION

The radioactivity observed in T_{90} serum probably reflects the difference between uptake and clearance of radioactive material from serum during the 90 min the experiment lasted. Clearance of protein complexes larger than 19S from the blood is proportional to its size (Christian and Lightfoot, 1970). These authors found a rapid clearance of labelled human albumin from the serum of rabbits immunized to this protein,

and a slower clearance in immune tolerant animals. In the present study, 28 per cent of the total serum radioactivity was associated with large complexes with a K_{av} of 0.06 (Fig. 1), and the rapid clearance reduced the serum radioactivity at T_{90} . The higher serum radioactivity in the immunized animals, compared with that in the non-immunized animals, may therefore be attributed to an increased uptake of radioactive material in the former.

The high ratio of counts/min/OD₂₈₀ and the size of the protein complexes indicated that peak I included antigenically intact labelled albumin which had formed soluble immune complexes.

Peak II exhibited a low ratio of counts/min/OD₂₈₀ and a close conformity between the OD₂₈₀ and scintillation profile. These observations are compatible with adsorption of labelled metabolites to the bulk of serum proteins. Antigenically unrelated albumin may also have contributed to the radioactivity of these fractions. The serum content of low molecular radioactive material ($K_{av} > 0.55$) reflects a rapid degradation of the labelled albumin and prevented interpretation of the autoradiographs.

The higher serum radioactivity found in immunized animals may reflect an increased permeability of the mucosal basement membrane. Basement membranes are generally considered as filtering structures for macromolecules. Complexes with a molecular size corresponding to a sedimentation coefficient of 19S or larger are entrapped in vascular and glomerular basement membranes (Cochrane and Hawkins, 1968; Kniker and Cochrane, 1968). Immunofluorescence studies of gingival tissue have shown the presence of IgG between the epithelial cells (Brandtzaeg and Kraus, 1965; Brandtzaeg, 1966) and the immunoglobulins and complement factor 3 have been detected in gingival fluid (Brandtzaeg, 1966; Shillitoe and Lehner, 1972).

Antibody to a topically applied horseradish peroxidase was observed in the extraneous cell coat and in the basement membrane of crevicular epithelium in an electron microscopic study by McDougall (1972). These studies suggest that serum proteins permeate the mucosal connective tissue and epithelium. Assuming a slow penetration of tritiated albumin through the epithelium, soluble immune complexes may have been formed in the presence of excess antibody; this may be analogous to the effect of a small amount of intravenously injected antigen (Cochrane and Hensom, 1970). Immune complexes formed in the mucosa may be trapped in its basement membrane where they can induce permeability changes. Gang, Mautner and Kalant (1970) gave rats an intravenous injection of rabbit antiserum to rat glomerular basement membranes and observed a 30 per cent loss of lipid phosphorus from the glomerular basement membranes within 30 min; the animals also showed proteinuria.

Apart from the effect on the basement membrane, the presence of antibodies with complement-fixing properties may promote diffusion of antigen through the mucosa by influencing the submucosal ground sub-

stance. Release of enzymes from leukocytes, attracted to the site through an immune complex-activated complement system, may change the gel-sol ratio of the ground substance to favour the sol part.

The results of the present study are in accordance with Bockman and Winborn (1966) who observed an increased absorption of horse ferritin through the intestinal mucosa of hamsters immunized by subcutaneous injections of ferritin emulsified in adjuvant. Walker and Isselbacher (1972), however, found a consistent and significant decrease in the intestinal absorption of horseradish peroxidase in rats which had been orally immunized with this antigen. This discrepancy may be due to the different immunization procedure. The oral route of immunization is considered to stimulate the synthesis of secretory IgA, which exerts its effect at the surface of the intestinal mucosa. It is likely that this would tend to reduce the amount of horseradish peroxidase actually penetrating the intestinal mucosa.

The higher serum radioactivity found in immunized animals in the present study does not necessarily imply an increased permeability of the mucous membrane. The formation of immune complexes superficial to the basement membrane might facilitate phagocytosis of the labelled albumin by leukocytes in the oral epithelium. Labelled metabolites released from these leukocytes may then penetrate the basement membrane and contribute to the higher total serum radioactivity in the immunized animals.

In order to determine if the increased uptake in immunized animals is specific to the antigen, or reflects a general increase in permeability, the penetration experiments will have to be repeated with a test solution containing a stable tracer molecule, such as labelled dextran, and an unlabelled antigen.

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Résumé—La pénétration de l'albumine humaine étiquetée à travers la muqueuse orale de cobaye a été évaluée par numération scintillante d'échantillons de sérum. On a observé une radioactivité plus élevée du sérum chez les animaux immunisés à l'albumine que chez les animaux non-immunisés. La chromatographie de gel du sérum révéla une dégradation substantielle de l'albumine, mais la précipitation immune confirma la pénétration de l'albumine intacte au point de vue antigénique. Les résultats suggèrent la possibilité d'une augmentation en perméabilité de la membrane basale muqueuse par déposition de complexes immunes.

Zusammenfassung—Es wurde die Durchdringung von gekennzeichnetem menschlichem Albumin durch orale Mukosa von Meerschweinchen durch Flimmernzählung von Serumproben geschätzt. Es wurde größere Serumradioaktivität bei, auf Albumin immunisierten Tieren als bei nicht immunisierten Tieren beobachtet. Gel-Chromatographie des Serums zeigte eine bedeutende Ablagerung von Albumin, aber immuner Niederschlag bestätigte Durchdringung von antigenisch intaktem Albumin. Die Ergebnisse legen die Möglichkeit einer Erhöhung der Permeabilität der mukosalen Basalmembran durch Ablagerung von immunen Komplexverbindungen nahe.