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IN VITRO SYNTHESIS OF IMMUNOGLOBULINS, SECRETORY COMPONENT AND COMPLEMENT IN NORMAL AND PATHOLOGICAL SKIN AND THE ADJACENT MUCOUS MEMBRANES

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SUMMARY

A study on the synthesis of immunoglobulins (IgG, IgA, IgM, IgD, and IgE), secretory component and complement in normal and pathological skin and in the adjacent mucous membranes (i.e. conjunctiva, nasal, oral and vaginal mucosa) is reported. The results are based on the culture of tissue samples in a medium with two radioactive amino acids and the detection of synthesized proteins by autoradiography of the immunoelectrophoretic pattern of the culture fluid, except in the case of IgE for which the Ouchterlony technique was used.

The results indicate that the normal skin does not synthesize immunoglobulins, whereas normal mucous membranes produce IgG and IgA. In the lesions of various skin diseases immunoglobulins are synthesized, mainly IgG but sometimes also IgA and IgE. The cells responsible for the production of immunoglobulins are plasma cells and lymphoid cells present in the skin lesions and mucous membranes.

Synthesis of the free secretory component could be demonstrated only in certain mucous membranes (i.e. conjunctiva, nasal mucosa, and oral mucosa).

Complement (C3) synthesis was found in normal skin, mucous membranes (i.e. conjunctiva, nasal and oral mucosa), and in the lesions of such skin diseases as discoid lupus erythematosus, (bullous) pemphigoid, dermatitis herpetiformis, malignant reticulosis, eczema and lichen planus. Complement production was also demonstrated in allergic skin reactions (i.e. tissue from allergic-positive patch tests, positive Mantoux tests and drug eruptions), but no immunoglobulin synthesis was detected in these lesions.

INTRODUCTION

The factors concerned in the defence against commensal and pathogenic bacteria are the intact integument (skin and mucous membranes), the humoral factors (immunoglobulins, the complement system, lactoferrin, lysozyme and other less well-defined antimicrobial serum factors), and certain cellular components (phagocytic cells and lymphocytes). In the

normal skin the defence mechanism is formed by the horny layer, constituting a mechanical barrier, and by the acid reaction of the skin surface effected by the free fatty acids in the sebaceous secretions and the lactic acid in the sweat (Marples, 1965; Ebling, 1970; Naylor, 1970; Humphrey & White, 1970). In the mucous membranes, which lack the horny layer, humoral and cellular factors play a more important role in this respect.

The local synthesis of immunoglobulins by several mucous membranes, including those of the respiratory digestive, and female genital tracts, has been studied by means of a tissue culture technique (van Furth & Aiuti, 1969; Aiuti *et al.*, 1970). At these sites, according to the results of immunofluorescence studies, immunoglobulins are synthesized by plasma cells and lymphoid cells (Brandtzaeg & Kraus, 1965; Crabbé & Heremans, 1966; Eidelman *et al.*, 1966; Brandtzaeg *et al.*, 1967; Heremans & Crabbé, 1967; Gelzayd *et al.*, 1967, 1968; Rossen *et al.*, 1968; Söltoft, 1969; Tourville *et al.*, 1969, 1970; Savilahti, 1972); the synthesis of complement was not included in these studies.

In various skin diseases such as lupus erythematosus, pemphigus, (bullous) pemphigoid and dermatitis herpetiformis, extracellularly located immunoglobulins and complement were demonstrated in the skin with the immunofluorescence technique (Burnham *et al.*, 1963; Cormane, 1964, 1967; Kalsbeek & Cormane, 1964; Cormane *et al.*, 1966, 1970; ten Have-Opbroek, 1966; Baart de la Faille-Kuyper & Cormane, 1968; Beutner *et al.*, 1968; Chorzelski & Cormane, 1968; Pohle & Tuffanelli, 1968; Chorzelski *et al.*, 1969; van der Meer, 1969, 1972; Cormane & Szabò, 1970; Cormane & Gianetti, 1971; Holubar *et al.*, 1971; Jordan *et al.*, 1971; Sams & Jordan, 1971), but it is not known whether the immunoglobulins and complement occurring in these skin lesions are synthesized locally or arise from the blood. Immunoglobulin-positive plasma cells have occasionally been found in the infiltrates of skin lesions of patients with lupus erythematosus (Hiroaki Ueki, 1968; Baart de la Faille-Kuyper, 1969). It is also not clear whether the immunoglobulins and complement in the skin have a deleterious effect or contribute to the healing of the lesions. These questions cannot be answered without more information about the sites of synthesis of immunoglobulins and complement located outside the lymphoid tissues.

The present paper describes a systematic study of the synthesis of these proteins by normal and pathological skin and adjacent mucous membranes, performed with an *in vitro* culture technique.

MATERIALS AND METHODS

Tissues

The samples of normal skin were obtained from ten individuals aged from 23 to 80 years and those of pathological skin from patients with various skin diseases, the ages of this group ranging from 19 to 91 years. At the time of sampling, the majority of the patients had not received any treatment for periods varying from weeks to months or years. In the few cases in which local treatment had been applied, this was stopped at least 3 days before the biopsy specimens were taken. Only some patients with malignant reticulosis and dermatitis herpetiformis were on systemic treatment with immunosuppressive drugs and sulphonamides, respectively, when the biopsies were performed.

Mucous membranes were obtained during surgery. The conjunctiva samples derived from patients aged from 3 to 76 years treated for strabismus or cataract, the samples of nasal mucosa from patients aged from 8 to 73 years requiring correction of a deviated nasal

septum, the oral mucosa samples (frenulum labii, gingiva) were also obtained during corrective surgery, and the vaginal mucosa material during surgery for vaginal prolapse.

Biopsies of tissues

Skin specimens were taken after local anaesthesia with 2% lydocaine (Xylocaine®, Astra-Blomberg N.V., Rijswijk, The Netherlands), with a biopsy punch (Dik, Blaricum, The Netherlands), without previous disinfection of the skin. Per patient, two or three samples with a diameter of 3–5 mm were taken from one lesion, or, when this was not possible, from two similar lesions. One sample with a diameter of 5 mm was used for culture study, the other one or two samples for immunofluorescent and histological examinations (Lai A Fat, Cormane & van Furth, 1973).

After aseptic removal, the tissues were placed in Petri dishes and processed with as little delay as possible (i.e. within 30 min).

Histological examination

Biopsy samples of skin were studied histologically to obtain confirmation of the clinical diagnosis and, in normal skin and mucous membranes, to determine whether abnormal cellular infiltrates were present. The samples were fixed in 10% formaldehyde and, after dehydration and embedding in paraffin, cut into 6-μm thick sections and stained with haematoxylin and eosin.

Cultivation of tissues

The method used for the study of immunoglobulin and complement synthesis *in vitro* has been described by Hochwald *et al.* (1961) and in detail by van Furth *et al.* (1966). Briefly, the procedure is as follows. After removal of the epidermis with a surgical blade, the skin specimen was minced in Hanks's balanced salt solution and the fragments were placed against the wall of a roller tube with a capillary pipette. The wet weight of the cultured skin tissue fragments varied from 80–130 mg and that of the mucous membranes from 30 mg (conjunctiva) to 200 mg (vagina). The tissue fragments were incubated for 48 hr at 37°C in 1 ml modified Eagle's medium in which lysine and isoleucine were replaced by 1 μCi/ml ^{14}C L-lysine (spec. act. 310 mCi/mmol, Schwarz Bio Research, Orangeburg, N.Y.) and 1 μCi/ml ^{14}C L-isoleucine (spec. act. 312 mCi/mmol, Schwarz Bio Research, Orangeburg, N.Y.), respectively. If the culture had not metabolized after 48 hr, which was checked by means of the indicator methyl red in the medium, the culture was incubated 24 hr longer. If there was still no metabolism after 72 hr of incubation, the culture was discarded. This occurred frequently at the beginning, when the skin had been disinfected with 70% ethyl alcohol before the biopsies were taken, but after this was omitted, it was rarely necessary to discard a culture. After incubation, the cultures were frozen (−20°C); after thawing, they were dialysed against phosphate buffer (0·015 M, pH 7·6) for 72 hr at 4°C, concentrated by lyophilization, and dissolved in 0·1 ml bidistilled water.

Analysis of culture fluid

The culture fluid was analysed by micro-immunoelectrophoresis (Scheidegger, 1955). Because concentrated culture fluids often contain too little protein to provide well-defined precipitation lines, a carrier serum was routinely added once to the antigen well (about 1·5 μl). After absorption of the serum into the agar, the culture fluid was added five times to the antigen well. The carrier serum was usually normal human serum, but for IgD we used the

serum of a patient with an IgD paraprotein, for secretory component casein-free and defatted human milk obtained on the seventh day post-partum (kindly supplied by Dr P. J. J. van Munster, Department of Paediatrics, University of Nijmegen), and for complement assay a 3-day-old normal human serum, stored at 4°C.

Electrophoresis was carried out at room temperature (2 hr, 3·5 V/cm). The antisera used were horse anti-human serum, horse anti-IgA, horse anti-IgM, rabbit anti-complement (i.e. anti-β_1C, anti-β_1A, anti-α_2D and anti-β_1E) (all obtained from the Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam), swine anti-IgD (Nordic Pharmaceutical and Diagnostics, Tilburg), and rabbit anti-secretory component serum, which contained antibodies against free and bound secretory component (van Munster *et al.*, 1971). This antiserum was kindly supplied by Dr P. J. J. van Munster, Department of Paediatrics, University of Nijmegen. The precipitation lines were developed for 24 hr at room temperature, after which the slides were washed for 72 hr in phosphate-buffered saline, dried, and stained with amido black.

The synthesis of IgE was investigated with the micro-Ouchterlony technique, because immunoelectrophoresis did not give reproducible precipitation lines. Five millilitres of a 1·3 per cent agar solution (Noble, Difco Laboratories, Detroit, Michigan, U.S.A.) in phosphate-buffered saline was poured on a slide (5 × 5 cm). The outer wells (inner diameter: 2 mm; content about 8 μl) were filled with a carrier serum with a high IgE level (kindly provided by Dr E. E. Reerink Brongers, Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam) and after absorption into the agar, culture fluid was added three times to the antigen well. The central well (inner diameter: 2 mm; situated 5 mm from the outer wells) was filled with rabbit anti-IgE serum (Central Laboratory of Red Cross Blood Transfusion Service, Amsterdam). The precipitation lines were developed for 24 hr at room temperature and then washed for 72 hr in phosphate-buffered saline, dried, and stained with amido black.

Evaluation of the synthesized proteins

The proteins synthesized *de novo* were detected and identified by autoradiography of the immunoelectrophoretic pattern of the culture fluid and of the Ouchterlony plate.

Autoradiography was performed at room temperature with film (Kodak RS PAN, 650 ASA) cut into strips to fit the slides. The exposure time ranged from 21 to 23 days. The films were developed with Rodinal solution (Agfa) diluted 1 to 10, for 10 min at 20°C.

The intensity of the autoradiographic lines, indicating the amount of protein synthesized, is classified according to a scale ranging from − = negative; (+) = just visible; + = clearly visible; to a maximum of + + + +, by comparison with a standard pattern. All readings were made independently by two observers.

The results are presented on the following basis: in each category of cultures the intensity of the autoradiographic line was scored (− = 0; (+) = 1; + = 2; + + = 3; + + + = 4; + + + + = 5), and the mean of the scores was calculated and expressed according to the classification described above.

RESULTS

Immunoglobulin synthesis

The analysis of immunoglobulin synthesis in the skin and mucous membranes gave the following results (Table 1).

TABLE 1. Immunoglobulin synthesis in normal skin and adjacent mucous membranes*

Origin of biopsy specimen	Total number of cultures	IgG		IgA		IgM		IgD		IgE	
		Number of positive cultures	Mean† intensity of all cultures	Number of positive cultures	Mean† intensity of all cultures	Number of positive cultures	Mean† intensity of all cultures	Number of positive cultures	Mean† intensity of all cultures	Number of positive cultures	Mean† intensity of all cultures
Normal skin	10	0	−	0	−	0	−	0	−	0	−
Conjunctiva	14	14	++	14	++	2	−	0	−	0	−
Nasal mucosa	11	9	+	11	+	4	−	0	−	0	−
Oral mucosa	14	14	+	10	(+)	0	−	0	−	0	−
Vaginal mucosa	24	20	(+)	9	−	0	−	0	−	0	−

* The intensity of the autoradiographic lines is classified from: − = negative, (+) = just visible, + = clearly visible, to a maximum of ++++.
† Calculated as described under Materials and Methods.

Normal skin

No synthesis of immunoglobulins was detected in normal skin. Histological sections revealed no abnormal cell infiltration.

Mucous membranes

All the cultures of conjunctiva showed synthesis of IgG and IgA (Fig. 1). The intensity of the lines was equal except in four cultures showing more IgA than IgG. IgM was found in only two of the cultures and then in small amounts.

The nasal mucosa synthesized IgG in all but two cultures, and IgA was always found. Five cultures produced more IgA than IgG. IgM was synthesized in four cultures in small amounts.

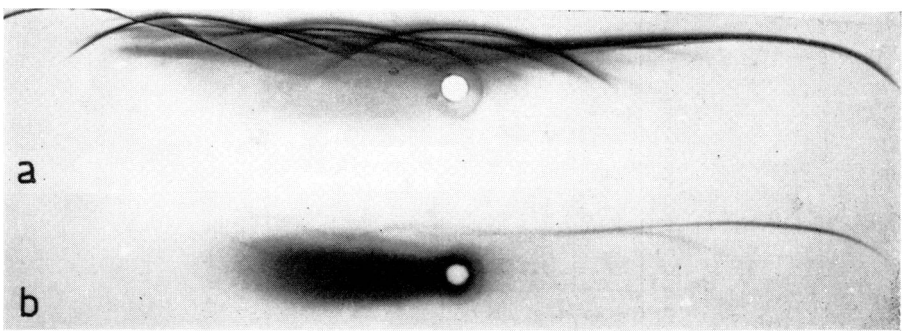

FIG. 1. Conjunctiva. (a) Immunoelectrophoretic pattern of carrier serum and culture fluid developed with horse anti-human serum. (b) Autoradiograph with clearly labelled IgG and IgA lines.

In the oral mucosa IgG was generally synthesized in distinct amounts, and in the majority of the cultures IgA was synthesized in smaller amounts than IgG. IgM production was not found in any of the cultures.

The majority of the cultures of vaginal mucosa synthesized IgG in small amounts, but only a minority produced IgA. IgM production was not detected.

None of the cultures of the mucous membranes showed synthesis of either IgD or IgE.

Histological examination of the mucous membranes demonstrated some scattered infiltration of lymphocytes and plasma cells, but no lymph follicles or signs of abnormal cell infiltration were present.

Pathological skin

The results of the histological examination of the biopsy samples of tissue taken to confirm the clinical diagnosis will be given in a subsequent publication together with the immunofluorescence findings concerning the cellular localization of immunoglobulin synthesis (Lai A Fat *et al.*, 1973). The results of the analysis of immunoglobulin synthesis are given in Table 2.

All but one of the skin cultures of patients with discoid lupus erythematosus showed the synthesis of small amounts of IgG, whereas IgA was found in only one of these cultures. IgG was also produced by the lesional skin of a patient with systemic lupus erythematosus. IgM, IgD and IgE were not detected.

In pemphigus, too, IgG was produced in small amounts, but the other four immunoglobulins were not synthesized.

In (bullous) pemphigoid IgG was formed consistently, mostly in small amounts, but in dermatitis herpetiformis only a minority of the cultures showed IgG synthesis. No other immunoglobulins were detected in either of these diseases.

The cultures of tissue from cases of mycosis fungoides always showed IgG synthesis, mostly in distinct amounts; IgA was found in seven out of twelve cultures, mainly in small amounts (Fig. 2). The synthesis of IgM and IgD could not be demonstrated, but IgE was found in three out of the twelve cultures. In the other forms of malignant reticulosis IgG was found in only three of the six cultures. In two of these positive cultures, both from patients with unclassifiable malignant reticulosis, IgA was produced, also in small amounts,

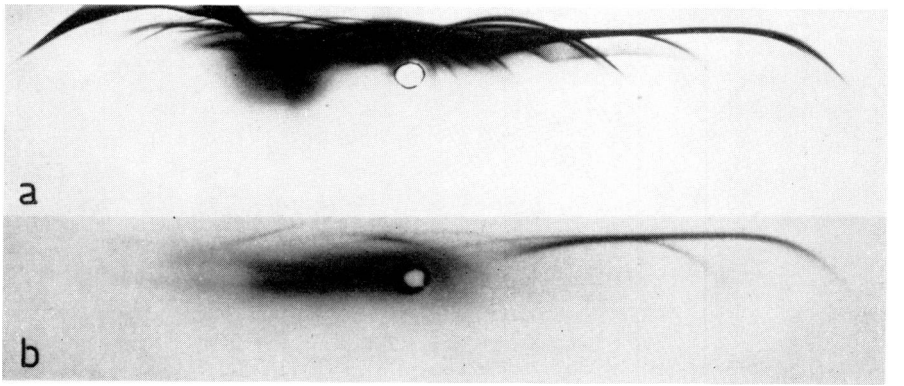

FIG. 2. Mycosis fungoides. (a) Immunoelectrophoretic pattern of carrier serum and culture fluid developed with horse anti-human serum. (b) Autoradiograph with clearly labelled IgG line and weakly labelled IgA line.

and in the third IgG-positive culture (from a patient with Brill–Symmers' disease) some IgM but no IgA was synthesized. IgD was not found, whereas IgE was synthesized in the two cultures in which IgG and IgA were demonstrated.

In the group of patients with eczema, IgG was synthesized in three of the cultures of atopic dermatitis and two cultures of nummular eczema, whereas IgA was found in only two of the cultures of atopic dermatitis. IgM and IgD were not demonstrable. IgE was produced in one culture of atopic dermatitis and two cultures of nummular eczema. These two IgE-positive cultures of nummular eczema showed no synthesis of IgG. Tissue from the lesions of the patient with stasis eczema and autosensitization showed the synthesis of IgG, IgA and IgE in distinct amounts (Fig. 5). The other types of eczema, i.e. seborrhoeic dermatitis and circumscribed neurodermatitis, showed no immunoglobulin synthesis.

In psoriasis vulgaris a small amount of IgG production could sometimes be demonstrated, but IgA was found in only one of these cultures. The other three immunoglobulins could not be detected.

The majority of the cultures of lichen planus synthesized only IgG, mostly in small amounts.

The lesional skin of a patient with lymphocytic infiltration (Jessner) produced a large amount of IgG as sole immunoglobulin.

TABLE 2. Immunoglobulin synthesis in lesions of various skin diseases*

Diagnosis	Total number of cultures	IgG		IgA		IgM		IgD		IgE	
		Number of positive cultures	Mean† intensity of all cultures	Number of positive cultures	Mean† intensity of all cultures	Number of positive cultures	Mean† intensity of all cultures	Number of positive cultures	Mean† intensity of all cultures	Number of positive cultures	Mean† intensity of all cultures
Immunological skin diseases											
Discoid lupus erythematosus	6	5	(+)	1	–	0	–	0	–	0	–
Systemic lupus erythematosus	1	1	(+)	0	–	0	–	0	–	0	–
Pemphigus vulgaris	1	1	(+)	0	–	0	–	0	–	0	–
Pemphigus foliaceus	1	1	(+)	0	–	0	–	0	–	0	–
(Bullous) pemphigoid	6	6	(+)	0	–	0	–	0	–	0	–
Dermatitis herpetiformis	9	4	(+)	0	–	0	–	0	–	0	–
Malignant reticulosis											
Mycosis fungoides	12	12	+	7	(+)	0	–	0	–	3	–
Other forms	6	3	(+)	2	--	1	–	0	–	2	–

Table 2 (*cont.*)

Eczemas											
Atopic dermatitis	5	3	(+)	2	–	0	–	0	–	1	–
Nummular eczema	4	2	(+)	0	–	0	–	0	–	2	(+)
Stasis eczema with autosensitization	1	1	+	1	(+)	0	–	0	–	1	+
Other eczemas	6	1	–	0	–	0	–	0	–	0	–
Miscellaneous group											
Psoriasis vulgaris	19	6	–	1	–	0	–	0	–	0	–
Lichen planus	9	6	(+)	0	–	0	–	0	–	0	–
Lymphocytic infiltration (Jessner)	1	1	++	0	–	0	–	0	–	0	–
Allergic skin reactions											
Allergic-positive patch test	11	2	–	0	–	0	–	0	–	0	–
Positive tuberculin reaction	8	1	–	0	–	0	–	0	–	0	–
Drug eruption	5	1	–	0	–	0	–	0	–	0	–

* The intensity of the autoradiographic lines is classified from: – = negative, (+) = just visible, + = clearly visible, to a maximum of ++++.
† Calculated as described under Materials and Methods.

In allergic skin reactions (i.e. test positive for mercury bichloride, potassium dichromate, nickel sulphate, dinitrochlorobenzene, neomycine, chloroacetamide, or shoe glue), positive Mantoux reactions to purified protein derivative (PPD), and in drug eruptions caused by penicillin, indomethacin, reserpin, or phenacetin, only small amounts of IgG were detected sporadically. The allergic-positive patch test areas from which the biopsy samples were taken were usually 48 hr old and the positive tuberculin reactions 48–72 hr old.

TABLE 3. Synthesis of secretory component (SC)*

Origin of biopsy specimen	Total number of cultures	Bound SC (IgA-SC)		Free-SC	
		Number of positive cultures	Mean† intensity of all cultures	Number of positive cultures	Mean† intensity of all cultures
Mucous membranes					
Conjunctiva	9	4	(+)	7	(+)
Nasal septum	9	5	(+)	9	+
Oral cavity	11	0	–	9	(+)
Vagina	15	1	–	2	–
Skin					
Normal	10	0	–	0	–
Mycosis fungoides‡	4	0	–	0	–
Unclassifiable malignant reticulosis‡	1	0	–	0	–
Eczema‡	2	0	–	0	–
Psoriasis vulgaris‡	1	0	–	0	–

* The intensity of the autoradiographic lines is classified from: – = negative, (+) = just visible, + = clearly visible, to a maximum of + + + +.

† Calculated as described under Materials and Methods.

‡ Cultures containing labelled IgA.

Synthesis of secretory component

The synthesis of secretory component (SC) was investigated in the cultures of normal skin and mucous membranes and in the skin cultures in which IgA was detected (Table 3).

The antiserum used gives different lines for free and bound SC (SC bound to IgA = IgA–SC) (Fig. 3). Labelling of the free SC line indicates *in vitro* synthesis of SC. Labelled bound SC lines in cultures containing labelled IgA could be due to *in vitro* binding of this protein to the SC of the carrier serum or of labelled SC of the culture fluid to IgA of the carrier serum, but it is also conceivable that both SC and IgA are synthesized and that these proteins combine *in vitro*. Since SC binds *in vitro* to polymeric IgA (Mach, 1970; Rádl *et al.*, 1971), the first and third of these three possibilities might indicate that the cultures synthesize IgA of this type. However, Jerry *et al.* (1972) recently demonstrated the binding of monomeric IgA2 genetic type $Am_2(+)$ to SC *in vitro*.

The results of the present studies show that radioactive bound SC was synthesized in about half of the conjunctiva cultures and free SC in the majority; the amount of free SC

was usually small. The nasal mucosa showed a similar pattern but with larger amounts of free SC than the conjunctiva. The cultures of the oral mucosa showed no bound SC, whereas free SC occurred in the majority of cultures. One culture of vaginal mucosa showed bound SC and two cultures free SC.

In the IgA-positive skin cultures for which culture fluid was still available, free and bound SC could not be detected (Table 3).

The absence of labelled IgA–SC lines in cultures containing radioactive IgA (i.e. oral mucosa, lesional skin) might be an indication that these tissues synthesize a monomeric form of IgA that does not combine with SC.

In normal skin the synthesis of free SC was not demonstrable.

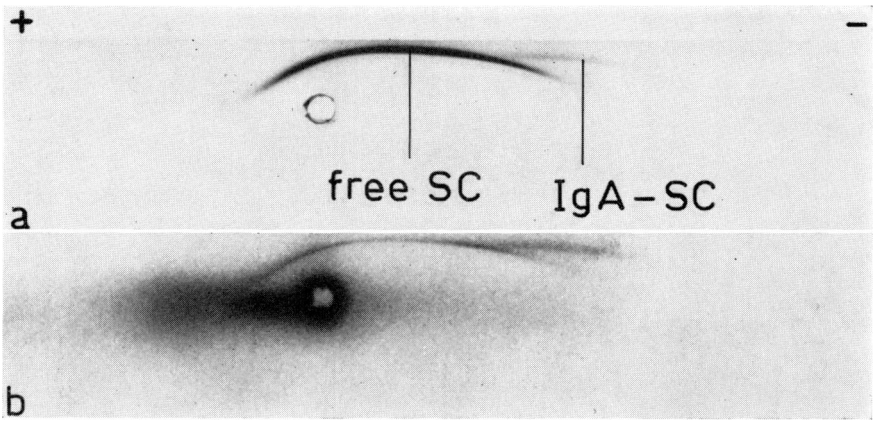

FIG. 3. Nasal mucosa. (a) Immunoelectrophoretic pattern of carrier and culture fluid developed with rabbit anti-SC serum. (b) Autoradiograph with labelling of free and bound SC line.

Synthesis of complement (*C*3, *C*4)

The synthesis of complement was investigated in all but a very few cases in which the supply of culture fluid was already exhausted.

The anti-complement serum used contained antibodies directed against C3 = β_1C, the C3 fragments β_1A and α_2D, and also antibodies against C4 = β_1E. Since C3 = β_1C converts rather rapidly into β_1A and α_2D at 37°C (within 48 hr; Wolters, 1972), any C3 synthesized in the cultures must have undergone similar conversion. Therefore, the synthesis of C3 can be deduced from the presence of one or more of the following labelled lines: β_1C, β_1A, α_2D.

The carrier serum used for complement assay was a 3-day-old serum held at 4°C. Immunoelectrophoretically, this serum showed the β_1C, β_1A and β_1E lines; the α_2D line was not demonstrable (Fig. 4, Ia).

In the present study (Table 4) half of the cultures of normal skin synthesized small amounts of C3.

The mucous membranes showed divergent patterns: conjunctival tissue synthesized C3 in all cultures, usually in distinct amounts (Fig. 4, Ib), but most of the nasal and oral mucosa cultures and some of the vaginal mucosa cultures showed the synthesis of only small amounts of C3.

In discoid lupus erythematosus C3 was synthesized in four out of six cultures; in lesional skin of systemic lupus erythematosus and pemphigus there was no synthesis of complement; in (bullous) pemphigoid C3 was produced in all cultures (Fig. 4, II); and in dermatitis herpetiformis all but one culture produced C3. In malignant reticulosis C3 was detected in the majority of the cultures. In psoriasis vulgaris C3 was synthesized occasionally, but in eczema this production was found for almost all of the cultures and in lichen planus for all of the cultures.

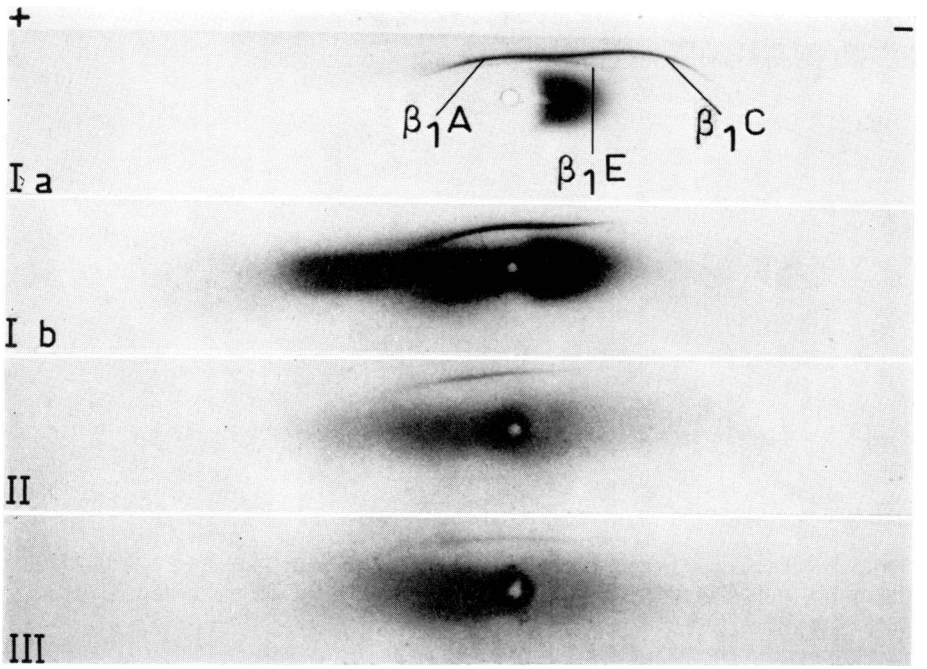

FIG. 4. Autoradiographs of immunoelectrophoretic patterns of carrier serum and culture fluids developed with rabbit anti-complement serum. (I) Conjunctiva. (a) Immunoelectrophoretic pattern. (b) Autoradiograph of (a) showing clearly labelled β_1A line and partially and weakly labelled β_1C line. (II) (Bullous) pemphigoid showing clearly labelled β_1A line and partially and weakly labelled β_1C line. (III) Allergic-positive patch test showing clearly labelled β_1A line.

In the allergic skin reactions, i.e. positive patch tests (Fig. 4, III) and positive Mantoux tests, C3 was detected in all of the cultures and in drug eruptions in four out of five cultures. The amount of C3 synthesized in the various skin diseases was small except for (bullous) pemphigoid, atopic dermatitis, lichen planus, and allergic-positive patch tests. The majority of the C3-positive cultures showed labelling of only the β_1A line, but sometimes part of the β_1C line was also labelled. The intensity of the β_1C line was weaker than that of the β_1A line.

The synthesis of C4 could not be detected in any of the cultures of normal skin, pathological skin, or mucous membranes.

Control experiments

The specificity of the method for the demonstration of immunoglobulin synthesis *in vitro*

TABLE 4. Synthesis of complement (C3, C4) in the skin and adjacent mucous membranes*

Origin of biopsy specimen	Total number of cultures	C3		C4	
		Number of positive cultures	Mean† intensity of all cultures	Number of positive cultures	Mean† intensity of all cultures
Normal skin	10	5	(+)	0	–
Mucous membranes					
Conjunctiva	13	13	++	0	–
Nasal septum	11	9	(+)	0	–
Oral cavity	14	10	(+)	0	–
Vagina	20	8	–	0	–
Skin diseases					
Immunological diseases:					
Discoid lupus erythematosus	6	4	(+)	0	–
Systemic lupus erythematosus	1	0	–	0	–
Pemphigus vulgaris	1	0	–	0	–
Pemphigus foliaceus	1	0	–	0	–
(Bullous) pemphigoid	6	6	+	0	–
Dermatitis herpetiformis	9	8	(+)	0	–
Malignant reticulosis:					
Mycosis fungoides	12	8	(+)	0	–
Other forms	6	4	(+)	0	–
Eczemas:					
Atopic dermatitis	4	4	+	0	–
Other eczemas	11	9	(+)	0	–
Miscellaneous group:					
Psoriasis vulgaris	14	6	–	0	–
Lichen planus	9	9	+	0	–
Lymphocytic infiltration (Jessner)	1	1	(+)	0	–
Allergic skin reactions					
Allergic-positive patch test	11	11	+	0	–
Positive tuberculin reaction	8	8	(+)	0	–
Drug eruption	5	4	(+)	0	–

* The intensity of the autoradiographic lines is classified from: – = negative, (+) = just visible, + = clearly visible, to a maximum of ++++.

† Calculated as described under Materials and Methods.

has been proven (van Furth *et al.*, 1966), but it was necessary to determine whether this would hold for the *in vitro* synthesis of secretory component and complement. For this purpose, the following control experiments were done (in duplicate).

Culture of dead tissue

Culture experiments with dead tissue were performed to investigate whether there is

adsorption of radioactive amino acids onto the complement and secretory-component molecules present in the tissues. A biopsy sample of nasal mucosa was divided in two parts, one of which was frozen at −20°C, thawed three times, and then incubated with radioactive medium for 48 hr at 37°C; the other part was cultured routinely. The autoradiographs of the culture fluid of the frozen tissues showed no labelled lines, whereas the other culture showed labelled IgG, IgA, IgA–SC, free SC, and complement (C3) lines.

Incubation of normal serum

To investigate non-specific adsorption of radioactive amino acids onto serum complement, 1 ml normal serum was incubated with 1 ml culture medium for 48 hr at 37°C and then treated as described for tissue cultures. Autoradiographs of the immunoelectrophoretic patterns of this serum showed no labelled lines.

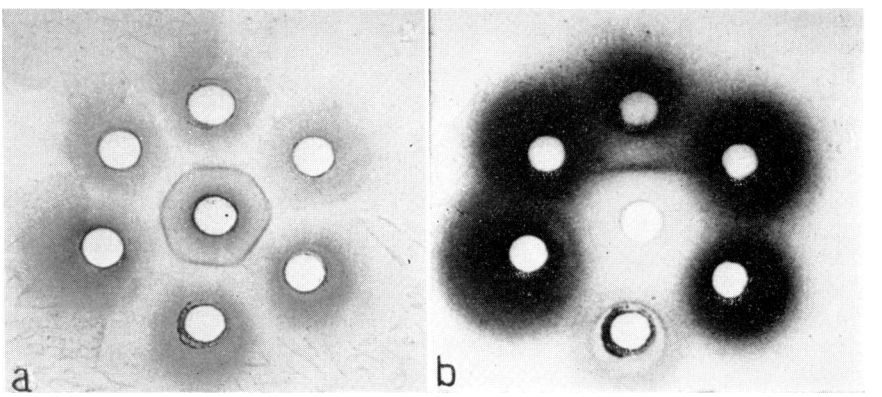

FIG. 5. (a) Ouchterlony plate: in central well, rabbit anti-IgE serum; in outer wells, culture fluid of various cultures. (b) Autoradiograph showing only one labelled IgE line in culture fluid from patient with stasis eczema and autosensitization (top well).

Incubation of hnman milk

To investigate the non-specific adsorption of radioactive amino acids onto SC, casein-free and defatted human milk was incubated with 1 ml culture medium for 48 hr at 37°C and then treated as described for tissue cultures. Autoradiographs of the immunoelectrophoretic pattern of this milk showed no labelled lines.

Culture with cycloheximide

The *de novo* synthesis of immunoglobulins, SC, and complement was also checked by adding cycloheximide, an inhibitor of protein synthesis, to cultures of nasal mucosa and conjunctiva.

Two cultures of equal weight were prepared from each biopsy sample and 10 μg cycloheximide per ml culture medium was added to one of them. The autoradiographic results ranged from very distinct inhibition to complete absence of immunoglobulin, SC and complement synthesis, whereas all of the routinely processed cultures synthesized these proteins in large amounts.

Culture with a medium containing ^{14}C-labelled methionine

Another way to demonstrate the specificity of SC synthesis is based on the fact that SC contains no methionine (Kobayashi, 1971; van Munster *et al.*, 1972).

A biopsy specimen of nasal mucosa was divided into two parts, one of which was cultured in a modified Eagle's medium in which the methionine was replaced by 2 μCi/ml ^{14}C L-methionine (spec. act. 255 mCi/Mmol); the other part was cultured in the routine medium (containing ^{14}C-L-lysine and ^{14}C L-isoleucine). The autoradiograph of the former culture showed no labelled SC line but did have labelled Ig lines, whereas the latter showed a very strongly labelled SC line as well as labelled Ig lines.

DISCUSSION

Several conclusions can be drawn from the present results. The normal skin does not synthesize immunoglobulins, but the mucous membranes produce these proteins, as do the lesions of several skin diseases. Free and bound SC are produced in certain mucous membranes and not in normal and pathological skin. Complement is synthesized in normal skin and mucous membranes as well as in the lesions of most of the skin diseases investigated.

The results of the control experiments show conclusively that the labelled immunoglobulins, SC and complement lines were not due to aspecific adsorption of radioactive amino acids onto these proteins during incubation *in vitro*. For IgG the actual incorporation of radioactive amino acids into this protein has been demonstrated by autoradiography of a fingerprint (van Furth *et al.*, 1966). Since this method was not used for SC and complement, binding of labelled proteins to SC and complement from the carrier serum remains possible although it is very unlikely, as shown for SC by the ^{14}C methionine experiment. It may be concluded that *de novo* synthesis of these radioactive proteins occurs *in vitro*.

The synthesis of IgE *in vitro* was demonstrated with the Ouchterlony technique. This test cannot be applied routinely for the detection of immunoglobulin synthesis, because other labelled proteins of the culture fluid can be adsorbed non-specifically onto the immune precipitate (van Furth, 1967). But the specificity of the labelling of the IgE line in this study is supported by two findings: first, that many of the cultures containing radioactive proteins (e.g. immunoglobulins, complement, or SC) remained negative for IgE with the Ouchterlony technique (Fig. 5); and secondly, that some of the cultures positive for IgE with this technique were also indicated to be positive by the autoradiographic analysis of the immunoelectrophoretic pattern.

The immunofluorescent technique demonstrates that the immunoglobulins synthesized in the skin are produced by plasma and lymphoid cells. In general, no immunoglobulin-positive cells were found in cultures of tissue specimens giving negative results in the synthesis studies (Lai A Fat *et al.*, 1973).

The present results concerning the synthesis of immunoglobulins in mucous membranes and various skin diseases are consistent with the general view that these proteins play an important role in the host defence of epithelia lacking a horny layer and probably also in skin lesions in which the barrier function of the horny layer is impaired, although here immunoglobulins may have a deleterious effect. As could be expected, in the normal skin with a horny layer and a normal barrier function, no immunoglobulin production could be detected.

Our findings concerning the *in vitro* synthesis of immunoglobulins by mucous membranes are not in complete agreement with the reports on immunofluorescence studies. In the nasal

mucosa, for example, we found IgG and IgA to be synthesized in about equal amounts, whereas Brandtzaeg *et al.* (1967) reported immunofluorescence studies in which IgA-positive cells were predominant. Other mucous membranes, i.e. of the bronchial wall, oesophagus, stomach, duodenum, appendix and colon, have also been reported to show equal synthesis of IgG and IgA (van Furth & Aiuti, 1969) in contrast to immunofluorescence observations by others (Crabbé *et al.*, 1965; Crabbé & Heremans, 1966; Martinez-Tello *et al.*, 1968; Tourville *et al.*, 1969) and the ratio of the immunoglobulins found in external secretions (Chodirker & Tomasi, 1963). Since these secretions contain proteolytic enzymes that can degrade the immunoglobulins, and since IgA bound to SC (so-called secretory IgA) is more resistant than IgG to this proteolytic action (Cederblad *et al.*, 1966; Tomasi & Calvanico, 1968) the ratio of immunoglobulin concentrations in excreta does not necessarily reflect the ratio of local synthesis.

In the skin diseases investigated, different patterns of immunoglobulin synthesis in the skin were found. What is the function of immunoglobulins synthesized locally? They may be antibodies directed against pathogenic agents in the skin, for instance the mycosis cells in the skin infiltrate seen in mycosis fungoides, the paramyxovirus-like structures demonstrated by various investigators in skin lesions of patients with lupus erythematosus (Prunieras *et al.*, 1970; Hashimoto & Thompson, 1970; Schmitt *et al.*, 1971), or an (altered) antigen in the skin such as is found in the basement membrane in lupus erythematosus, (bullous) pemphigoid, dermatitis herpetiformis, and the intercellular substance of the epidermis in pemphigus. Another possibility is that the synthesized immunoglobulins are directed against irrelevant antigens, since lymphocytes are randomly seeded into an inflammatory lesion (Jasin & Ziff, 1969).

It is noteworthy that all the cases of mycosis fungoides showed immunoglobulin synthesis: IgG invariably, IgA frequently, and sometimes also IgE. Furthermore, some of the patients with mycosis fungoides who were on immunosuppressive drugs (corticosteroids or cytotoxic drugs) when the biopsy samples were taken for the culture studies, still synthesized immunoglobulins in the skin, albeit generally in smaller amounts than were found for the untreated patients. It is also interesting to report that when mycosis fungoides transformed into a reticulum cell sarcoma, as we have seen occur in two of our patients, no immunoglobulin synthesis could be demonstrated in the lesions.

The synthesis of immunoglobulins could not be demonstrated in all of the skin diseases investigated, possibly due to production below the level of detection (0·2 ng labelled IgG gives a just visible line on the autoradiograph; van Furth, 1966) because there were too few plasma cells and lymphocytes in the infiltrates. It is also conceivable that the lymphoid cells in the infiltrates are T-lymphocytes (thymus-derived lymphocytes), which have not been shown to synthesize one of the known immunoglobulin classes. T-lymphocytes are involved in allergic skin reaction (e.g. positive patch tests, tuberculin tests, and certain drug eruptions), and it was in these lesions that no immunoglobulin synthesis could be demonstrated. All these cultures showed the synthesis of complement (C3), but this could be expected, because mononuclear phagocytes are also involved in the allergic skin reactions.

Mononuclear phagocytes are known to produce certain complement factors (see review by Stecher, 1970; Schultz, 1971; Ruddy *et al.*, 1972; van Furth, 1973), but synthesis by other cells remains possible. Synthesis by mononuclear phagocytes is supported by the finding that in monocytic leukaemia peripheral blood leucocytes produce C3 (unpublished observations). A study with purified human monocytes and macrophages of different organs might provide

more evidence on this point. The biologic activity of complement components synthesized *in vitro* also needs further investigation, since many of the complement studies performed so far have demonstrated the synthesis of complement factors as proteins but have thrown little or no light on the function of these factors.

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