reported by Raman and Bierwaltes (1959) from an area further to the east of the present area of study.

The high P.B.^{131}I at 24 hours observed in this study both in goitrous deafmutes and in euthyroid persons with endemic goitre is of interest. It is apparently due to enhanced hormonal release—a reflection of thyroidal adaptation to iodide deficiency. The intense epithelial hyperplasia observed in Himalayan endemic goitre together with a lack of the usual dilution effect of thyroidal iodide are probably responsible for the rapid transit of iodine through the thyroid gland (Roy et al. 1964).

We have no evidence to substantiate the view of Clements (1958) that endemic deafmutism may be the result of genetic metabolic errors in the synthesis of thyroid hormones. All the subjects in the present study had avid goitres. They did not manifest a peroxidase defect as in Pendred's syndrome (Morgans and Trotter 1958). The absence of iodotyrosines in the blood and urine and the normal ratio of B.E.^{131}I to P.B.^{131}I indicate that some at least of the known metabolic defects of sporadic goitrous cretinism are unlikely to be present in our endemic deafmutes. Nevertheless, in the absence of direct tissue analysis, the evidence presented here as well as that of Bastenie et al. (1962) should be regarded as inferential rather than conclusive.

The similarity in the metabolic pattern between endemic deafmutes on the one hand and euthyroid persons with endemic goitre and euthyroid relatives of deafmutes on the other, also weakens the notion that endemic deaf-mutism (and cretinism) are causally related to endemic goitre and result from the operation of severe endemic influences over a long period. From the data presented here their responses can be regarded only as a reflection of iodide deficiency in an environment common to all three groups.

Our findings do not, however, rule out the possibility that endemic cretinism and deafmutism may result from damage to the fœtus at a critical stage of development by the same deficiency that is responsible for endemic goitre —namely, an adequate supply of iodide (Trotter 1960, Stanbury and Ramalingaswami 1963). The characterisation of the deafness as perceptive and the failure of speech development are compatible with this view. The acoustic nerve, the auditory apparatus, and the thyroid develop early in embryonic life (Batsakis and Nishiyama 1962). The thyroid develops first. Decreased thyroid activity produced by injecting thiourea into fertile developing chick-eggs has been found in association with concomitant changes in the sensory supporting cells of the acoustic papilla (Ramanoff and Lanfer 1956, Ritter and Lawrence 1960). Although the thyroid undergoes compensatory enlargement through the action of thyroid-stimulating hormone, resulting in a euthyroid state, the loss of hearing would be permanent. Himalayan endemic goitre is due to severe environmental deficiency of iodide. The deficiency is so severe that, in the life line of the thyroid, the gland rarely has an opportunity to involute and the thyroid at birth is hyperplastic, grossly enlarged, and poor in organic iodine (Roy et al. 1964). We believe that in this milieu appropriate conditions exist for the development of deafmutism, but the precise mechanism is still obscure.

Summary

A group of twenty deafmutes with endemic goitre in the Himalayas and nine of their close relatives were tested for thyroidal uptakes of ^{131}I, serum-P.B.^{127}I, serum-P.B.^{131}I, and B.E.^{131}I, 24 hours after receiving a tracer dose, for thyroidal discharge of trapped iodide after perchlorate or thiocyanate of potassium, and for iodotyrosines in serum and urine by means of chromatography.

The deafmutes gave results closely similar to those of euthyroid persons with endemic goitre from the same area—namely, raised thyroidal uptake of ^{131}I, low serum-P.B.^{127}I, high serum-P.B.^{131}I expressed as a percentage of administered dose in 24 hours per litre, absence of thyroidal discharge after thiocyanate or perchlorate of potassium, and absence of iodotyrosines in serum and urine. Thus, some of the enzymic defects observed in sporadic goitrous cretinism and deafmutism were not demonstrable in endemic deafmutes. Himalayan endemic deafmutism seems likely to be related to the endemic influences that lead to goitre, but the precise mechanism is unknown.

REFERENCES

Bastenie, P. A., Eramus, A. M., Thys, O., Beckers, C., van den Schrieck, H. G., de Visscher, M. (1962) *J. clin. Endocrin.* **22,** 187.
Batsakis, J. B., Nishiyama, R. H. (1962) *Arch. Otolaryng., Chicago,* **76,** 401.
Clements, F. W. (1958) *Bull. Wld Hlth Org.* **18,** 175.
de Quervain, F., Wegelin, C. (1936) *Der Endemische Kretinismus.* Berlin.
Greenwald, I. (1959) *Arch. Otolaryng.* **70,** 541.
McCarrison, R. (1908) *Lancet,* ii. 1275.
Morgans, M. E., Trotter, W. R. (1958) *ibid.* i, 607.
Murray, M. M., Ryle, J. A., Simpson, B. W., Wilson, D. G. (1948) *Medical Research Council Memorandum:* no. 18. London.
Ramalingaswami, V., Subramanyan, T. A. V., Deo, M. G. (1961) *Lancet,* i, 791.
Raman, G., Bierwaltes, W. H. (1959) *J. clin. Endocrin.* **19,** 228.
Ramanoff, A. L., Lanfer, H. (1956) *Endocrinology,* **59,** 611.
Ritter, F. N., Lawrence, M. (1960) *Laryngoscope,* **70,** 393.
Roche, J., Michael, R., Wolf, W., Nunez, J. (1956) *Biochim. Biophys. acta,* **19,** 308.
Roy, S., Deo, M. G., Ramalingaswami, V. (1964). *Amer. J. Path.,* **44,** 839.
Silver, S., Fieber, M. H., Yohalem, S. B. (1952) *Amer. J. Med.* **13,** 725.
Stanbury, J. B., (1960) *in* Metabolic Basis of Inherited Disease. New York.
— Litvak, J. (1957) *J. clin. Endocrin.* **27,** 654.
— Ramalingaswami, V. Iodine in Nutrition: A Comprehensive Treatise. New York (in the press).
Stott, H., Bhatia, B. B., Lal, R. S., Rai, K. C. (1931) *Indian J. med. Res.* **18,** 1059.
Taurog, A., Chaikoff, I. L. (1957) *in* Methods in Enzymology; vol. II, p. 836. New York.
Trotter, W. R. (1960) *Brit. med. Bull.* **16,** 92.
Wespi, H. J. (1945) *Schweiz. med. Wschr.* **75,** 625.

"BOUND" COMPLEMENT IN THE SKIN OF PATIENTS WITH CHRONIC DISCOID LUPUS ERYTHEMATOSUS AND SYSTEMIC LUPUS ERYTHEMATOSUS

G. L. KALSBEEK   R. H. CORMANE
M.B. Utrecht   M.D. Leyden

OF THE DEPARTMENT OF DERMATOLOGY,
UNIVERSITY OF UTRECHT, THE NETHERLANDS

AT about the same time and independently of each other, Burnham, Neblett, and Fine (1963),* and Cormane (1963) † were able to show with the direct immunofluorescent method, using commercially prepared fluorescein-labelled goat anti-human globulin and fluorescein-labelled goat anti-human gamma-globulin, a staining of the region of the basal membrane in cryostat sections of skin lesions of patients with lupus erythematosus.

The specificity of the phenomenon can be demonstrated by generally accepted procedures (Nairn 1962).

However, Cormane (1964) found no specific staining of the region of the dermal-epidermal junction in conditions which may be confused with lupus-erythematosus eruptions (e.g., polymorphic light eruptions and seborrhœic dermatitis), whereas according to the investigations of Burnham et al. gamma-globulin localisation at the dermal-epidermal junction can be demonstrated not only in lupus

---
* Presented at the 24th annual meeting of the Society for Investigative Dermatology Inc., Atlantic City, June 20, 1963.
† Presented at the 167th meeting of the Dutch Society of Dermatologists, Utrecht, Nov. 16, 1963.

erythematosus but also, though less conspicuous, in various other dermatoses (psoriasis, contact dermatitis, atopic dermatitis, eczematous dermatitis, nummular eczema, eczema solare, seborrhœic dermatitis, and erythroderma associated with mycosis fungoides).

However, in lupus erythematosus the immunological selectivity of the phenomenon could be established by selectivity tests (Burnham et al. 1963, Cormane 1964): the binding of gamma-globulin in the region of the basal membrane does not necessarily prove that an antigen-antibody reaction has occurred. If, however, the gamma-globulin is localised because of a reaction with antigen in situ, the concomitant binding of complement is to be considered.

Previous investigations have shown that complement is involved in the auto-immune mechanism underlying lupus erythematosus.

In vivo, the complement titre in the serum of patients with systemic lupus erythematosus appeared to be decreased (Elliott and Mathieson 1953).

Moreover, Dausset (1957), Gajdusek (1957), and Seligmann (1958) were able to demonstrate complement-binding antibodies against different organs and cells in the serum of patients with systemic lupus erythematosus.

In in-vitro experiments Robbins et al. (1957) and Hijmans and Schuit (1959) showed by complement-fixation tests that, in the lupus-erythematosus phenomenon, complement is bound to the nucleus-anti-nucleus complex.

With the fluorescent-antibody technique Feltkamp (1962) showed that in the antinuclear factor phenomenon complement was bound at the cell nucleus.

Lachman et al. (1962) and Morse et al. (1962) could demonstrate complement concomitantly bound with gamma-globulin at the sites of histological lesions in the renals and spleen of patients with systemic lupus erythematosus.

On the chance that complement might be concomitantly bound with gamma-globulin to the basal membrane, we have exposed cryostat sections of lupus-erythematosus lesions to fluorescein-labelled rabbit anti-human complement.‡

Method

Anti-human complement serum as described by Pondman and Peetoom (1961) was labelled with fluorescein-isothiocyanate.§ This serum contains antibodies against the complement factors C′3a and C′4.

Biopsy samples were obtained from the diseased and the normal skin of patients with discoid lupus erythematosus and systemic lupus erythematosus. The material was instantly frozen with CO_2 and transferred to a cryostat cabinet kept at $-20°C$ in which sections were cut on a microtome at 8 μ thickness and mounted on slides.

Before being stained, the slides were washed in phosphate-buffered saline pH 7·2 to remove any unbound serum components. Afterwards each section was covered with a few drops of fluorescein-labelled anti-

‡ The conjugate was kindly supplied by T. E. W. Feltkamp (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam; Director: Prof. Dr. J. J. van Loghem).
§ By Feltkamp (1964).

human complement diluted 1/30. Duplicate sections and sections of normal skin were similarly treated with: (a) commercially prepared fluorescein-labelled anti-human globulin from goat (Difco) or horse (Roboz); (b) unconjugated 1/30 diluted anti-human complement (Central Laboratory of the Blood Transfusion Service); and (c) commercially prepared unconjugated anti-human globulin from rabbit (Hoechst) or horse (Roboz).

After thirty minutes' incubation at room temperature, the sections were washed with phosphate-buffered saline pH 7·2, and mounted in F.T.A.-mounting fluid (Difco). The mounted sections were examined microscopically using a mercury super-pressure lamp (Osram HBO 200 W) as the source of ultraviolet light; UG 1 (3 mm.) and UG 2 (2 mm.) as "exciting" filters; and BG 23 (red) and GG 4 (UV) as "barrier" filters (Zeiss equipment).

Results

After exposure to fluorescein-conjugated anti-human complement serum and anti-human globulin serum all sections of the diseased skin from 8 patients (discoid 6, systemic 2) showed a well-demarcated zone of bright yellow-green fluorescence at the region of the basal membrane (also extending around the hair follicles). The dermis and the epidermis show the same fluorescence as the sections of the uninvolved skin.

The conspicuousness of the fluorescence zone in "lupus erythematosus" sections after staining with the above conjugates is not, however, the same in corresponding sections of different patients. Presumably there is a relation between the age of the untreated lesion and the immunofluorescent findings. Burnham et al. (1963) record a case in which an older facial lesion showed fluorescence of the "basal membrane" but a new arm lesion did not.

Furthermore, we had the impression that, in duplicate sections, a smaller fluorescent zone was seen after staining with fluorescein-labelled anti-human complement than after staining with fluorescein-labelled anti-human globulin. But obviously, from this kind of experiment, no conclusions can be drawn about the quantitative ratio of complement and globulin found in the region of the dermal-epidermal junction.

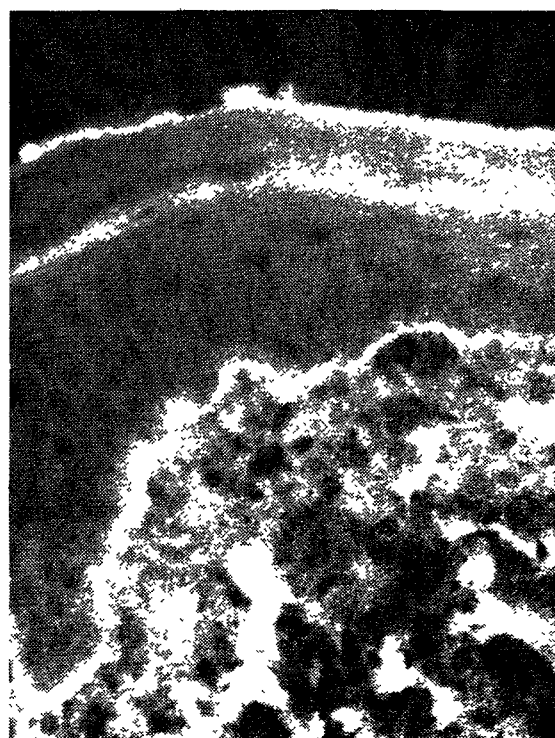In various dermatoses (e.g., psoriasis, lichen ruber,

Fig. 1—Chronic discoid lupus erythematosus: "diseased" skin. Note the specific staining of the region of the basal membrane.

Fig. 2—Chronic discoid lupus erythematosus: uninvolved skin. Note the absence of staining of the region of the basal membrane.

eczematous dermatitis, atopic dermatitis, seborrhœic dermatitis) Burnham et al. (1963), after staining with fluorescein-labelled anti-human globulin, observed an ill-defined bright yellow-green fluorescent band at the dermal-epidermal junction.

We, too, have seen this; but it clearly differed from the specific staining of lupus erythematosus (a) in its inconstant occurrence and (b) in its different morphological picture. In our opinion the poorly demarcated fluorescent band at the dermal-epidermal junction in psoriasis, as presented by Burnham et al. (1963), is the result of staining not of the dermal-epidermal junction but of the basal parts of the basal layer cells.

Since it was also seen after staining with unrelated fluorescein-labelled rabbit antisera, it cannot be considered specific.

Immunological Specificity

Evidence of the specificity of the staining with fluorescein-labelled anti-human complement can be provided as follows:

When sections of diseased skin were previously exposed to unconjugated anti-human complement, no fluorescence of the region of the basal membrane was seen, owing to blocking of the specific sites.

Neutralisation of the fluorescein-labelled anti-human complement with equal amounts of human complement removes its ability to produce specific staining.

At the moment we have no unrelated fluorescein-conjugated anti-complement sera with which to perform direct staining and the so-called sandwich technique to complete the specificity tests.

Burnham et al. (1963) were unable to show a concomitant local complement binding with guineapig complement and fluorescein-labelled rabbit anti-guineapig serum. We regard this as evidence of the specificity of our anti-human complement staining procedures.

Previous exposures of representative sections alternately to unconjugated anti-human globulin and unconjugated anti-human complement and subsequent staining with fluorescein-conjugated anti-human complement or fluorescein-labelled anti-human globulin respectively, does not influence the staining results. This means that these conjugates possess different binding sites on their molecules for the basal membrane.

This phenomenon has been confirmed by Feltkamp and Pondman (1964), who studied the immune complex of insulin/anti-insulin.

Summary

These results show that, in the skin lesions of discoid and systemic lupus erythematosus, anti-human complement (factors C′3a and C′4) and anti-human globulin are concomitantly bound to the region of the basal membrane.

In both forms of lupus erythematosus, therefore, the localised (gamma) globulin is probably immunological in character. The phenomenon does not occur in various other dermatoses (e.g., psoriasis, atopic dermatitis, eczematous dermatitis, seborrhœic dermatitis).

Hence the use of fluorescein-labelled (gamma) globulin in combination with fluorescein-labelled anti-human complement seems appropriate for diagnostic purposes, especially in cases of doubt, as already suggested by Cormane (1964).

REFERENCES

Burnham, T. K., Neblett, T. R., Fine, G. (1963) *J. invest Derm.* **41**, 451.
Cormane, R. H. (1964) *Lancet*, i, 534.
Dausset, J. (1957) *Vox Sang.* **2**, 225.
Elliott, J. A., Mathieson, D. R. (1953) *Arch. Derm. Syph.*, Chicago, **68**, 119.

IMMUNOLOGICAL SIGNIFICANCE OF THYMUS
DELAYED SKIN REACTION IN THYMECTOMISED RAT

MARIANO MESSINI
M.D. Padua
PROFESSOR OF SYSTEMATIC MEDICAL THERAPY, AND DIRECTOR OF THE POSTGRADUATE SCHOOL FOR LIVER AND METABOLIC DISEASES IN THE UNIVERSITY OF ROME

GIUSEPPE P. CENCI   GIANCARLO CUCCHI
M.D. Rome   M.D. Rome

IN studies of lymphoid organs, dating from 1931, Messini and coworkers found that in various metabolic conditions the thymus, lymph-nodes, and lymphoid tissue of the spleen play a similar part, the thymus being of prime importance (Messini 1931, 1933, 1958, Coppo 1931, Travia 1936a and b). This metabolic role of the thymus is now seen to be paralleled by its immunological role (Dougherty and White 1943, Dougherty 1952, Medawar 1957, Metcalf 1958, 1959, Burnet 1959, Miller 1961, 1963, Holmes and Burnet 1963). It is interesting to recall that as long ago as 1907 Frugoni found lymphocyte and plasma-cell accumulations in the muscles of patients with myasthenia.

We have studied the delayed skin reaction in rats thymectomised shortly after birth. This reaction is known to depend on the activity of immunologically competent cells (Landsteiner and Chase 1942, Cenci et al. 1952, Lawrence 1959).

Material and Methods

The thymus was removed from 99 hybrid Sprague-Dawley rats two to five days after birth. Hypothermia was induced by placing them, in a plastic container, in a refrigerator at about −10°C for twenty to twenty-five minutes. After removal of the thymus, the animal was reanimated under a 100-watt lamp and returned to its mother. The mortality of the procedure was about 50–55%.

Sensitisation.—At the age of 5–6 weeks the thymectomised rats were sensitised by an injection of 0·5 mg. crystalline bovine serum-albumin (Armour) in 0·10 ml. of Freund's adjuvant (solution A). On the twentieth day after sensitisation, they were skin-tested with 30 μg. of the serum albumin in 0·10 ml. physiological saline solution (solution B). This injection was given into one flank, while 0·10 ml. saline was injected into the other flank as control. The skin reaction was examined after twenty-four, forty-eight, and seventy-two hours, and its intensity recorded by conventional signs from 0 to + + + +.

Inflammation caused by chemical agents.—A small number of thymectomised and control rats received an intracutaneous injection of 0·10 ml. of a 0·5% solution of Evans blue into one flank and of 0·50 ml. of turpentine (diluted 1/20 in olive oil) into the other. The spread of the dye and the inflammatory reaction were examined at various times in the next twenty-four hours.

DR. KALSBEEK, DR. CORMANE: REFERENCES—*continued*

Feltkamp, T. E. W. (1962) *in* A report to the Foundation for Research on Rheumatic Diseases; Dr. Jan van Breemen Fund.
— Kruyff, K., Ladiges, N. C. J. J., Rümke, P. (1964) *Trans. N.Y. Acad. Sci.* (in the press).
— Pondman, K. W. (1964) Personal communication.
Gajdusek, D. C. (1957) *Nature, Lond.* **179**, 666.
Hijmans, W., Schuit, H. R. E. (1959) *Vox Sang.* **4**, 476.
Lachmann, P. J., Müller-Eberhard, H. J., Kunkel, H. G., Paronetto, F. (1962) *J. exp. Med.* **115**, 63.
Morse, J. H., Müller-Eberhard, H. J., Kunkel, H. G. (1962) *Bull. N.J. Acad. Med.* **38**, 641.
Nairn, R. C. (1962) Fluorescent Protein Tracing. Edinburgh and London.
Pondman, K. W., Peetoom, F. (1961) Proceedings of VIII Congress European Society of Hæmatology, p. 497.
Robbins, W. C., Holman, H. R., Deicher, H., Kunkel, H. G. (1957) *Proc. Soc. exp. Biol. Med., N.Y.* **96**, 575.
Seligmann, M. (1958) *Rev. franç. Étud. clin. biol.* **3**, 558.