As Table 2 shows, the highest concentrations of T-α-G in the healthy subjects were found in extracts of organs rich in lymphoid tissue and also in sperm. Since lymphoid tissue cells are present in many organs, it can be postulated that the main source of T-α-G is lymphoid tissue.

T-α-G is thus a tissue antigen linked primarily with lymphoid tissue and which, in certain pathological states when the immune status of the patient is disturbed, is found in increased quantities in the blood.

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SOME ASPECTS OF THE REACTION OF HEALTHY HUMAN BLOOD SERUM WITH EPIDERMIS

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KEY WORDS: normal antibodies; epidermis.

In some forms of pathology of the skin antibodies against certain antigens of the epidermis characteristic of the particular disease appear in the patients' blood. For instance, in lupus vulgaris a high titer of antibodies against antigens of intercellular adhesive substance is observed [7], in bullous pemphigoid there is a high titer of antibodies against antigens of the basement membrane [13], and the sera of patients with burns react with antigens of cells of the stratum basale of the epidermis [5]. Meanwhile there have been only isolated reports of the reaction of healthy human serum with antigens of cells of the epidermis [5]. Accordingly, the object of the present investigation was to study by the indirect immunofluorescence method the reaction of healthy human serum with the epithelium of the skin.

EXPERIMENTAL METHOD

The reaction of the sera of 70 clinically healthy subjects (blood donors) with sections of fetal and adult (aged 20-25 years) human skin was studied by the indirect immunofluorescence methods. Pieces of skin tissue taken from the region of the chest were freed from subcutaneous cellular tissue and frozen in petroleum ether, cooled in a mixture of acetone and dry ice to −76°C. Unfixed frozen sections were treated with serum (dilution from 1:4 to 1:20) for 18 h at 4°C. After rinsing for 20 min in running buffered physiological saline, pH 7.2, the sections were incubated for 45 min with fluorescein isothiocyanate-labeled antibodies against human IgG. Antibodies were isolated from donkey antiserum by means of an immunosorbent containing human IgG treated with glutaraldehyde. In control experiments, skin sections were treated with human IgG (concentration 500 μg/ml) for 18 h at 4°C, after which the sections were incubated with luminescent antibodies against human IgG. To study the organ specificity of the reaction the sera were first absorbed with a suspension of epithelial cells from human fetal skin, a suspension of keratinized epidermal cells, and a homogenate of tissues of certain organs (liver, kidney, heart, brain, spleen). The suspension of fetal epidermal cells was obtained by treating skin with 1% trypsin solution for 3 h at 37°C. The suspension of keratinized cells from adult human epidermis was obtained by mechanical stripping of the surface layers of the epithelium. The serum and suspension of epidermal cells or homog-

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enate of tissues from other organs, taken in the ratio of 2:1, were incubated for 1 h at 37°C and overnight at 4°C.

EXPERIMENTAL RESULTS

When sections of human skin were treated with blood serum from clinically healthy subjects in a dilution of 1:20 intense fluorescence of the cell cytoplasm in the differentiated layers of the epidermis was observed in 100% of cases (Fig. 1). The absence of a reaction in the intercellular spaces and the clearly defined cell boundaries enabled changes in the shape and orientation of the cells to be placed at the boundary of one layer and another in the course of differentiation. Healthy human serum reacted less strongly with the cytoplasm of cells of the stratum spinosum than with cells of the stratum granulare and, in particular, the stratum corneum; the intensity of fluorescence of the cell cytoplasm in the differentiated layers of the epidermis thus increased in the course of their keratinization. Accordingly, during titration of the normal serum on skin sections fluorescence of the cell cytoplasm disappeared first in the stratum spinosum, but as dilution proceeded, the reaction with cells of the stratum granulare, and later with the stratum corneum of the epidermis successively weakened and disappeared. The results of titration of the 15 most active sera showed that the reaction with the cell cytoplasm in the stratum spinosum continued as far as a dilution of 1:40, and with cells of the stratum corneum to a dilution of 1:100-1:120. The sera reacted with epithelium of human fetal skin in rather higher dilutions — as far as 1:200. In 43% of cases the healthy human sera also reacted with the perinuclear zone of differentiated cells of the epidermis; under these circumstances two zones of fluorescence were clearly distinguishable in their cytoplasm: the perinuclear zone and the cytoplasm proper (Fig. 1b). The sera reacted with antigens of the perinuclear zone to a dilution of 1:20. In 20% of cases, when the skin sections were treated with healthy human serum, weak and diffuse fluorescence of the cell cytoplasm was observed in the stratum basale of the epidermis. This reaction was observed with normal serum in a dilution of up to 1:16.

Preliminary absorption of the serum by a suspension of human fetal or adult epidermal cells completely abolished its reaction with epithelial cells of human fetal and adult skin. Absorption of the serum with the homogenate of tissues from other organs did not affect the intensity of the reaction of the serum with epidermal cells.

When sections of human fetal and adult skin were treated with the preparation of human IgG in a concentration of 500 μg/ml diffuse fluorescence of the cytoplasm of the differentiated epidermal cells was observed, but the reaction was weaker than that with whole serum. Just as when the skin sections were treated with human serum, an increase in the intensity of fluorescence of the cytoplasm was observed in cells of the differentiated layers in the course of their keratinization. The results thus show that blood serum from healthy subjects reacts in 100% of cases with a cytoplasmic component of differentiated epidermal cells and that this reaction is still found in tests with IgG isolated from these sera. The immunomorphologic picture of the reaction of normal serum with the epithelium of the skin is distinguished by great constancy and by intense fluorescence of the cytoplasm of the differentiated epidermal cells, whereas the reaction with the cells of the stratum basale is weak and comparatively rarely observed. In a high proportion of cases healthy human blood serum reacts not only with the cytoplasm, but also with the perinuclear zone, although the more intense fluorescence of the cytoplasm proper in the differentiated cells makes it more difficult to detect the reaction with antigens of the perinuclear zone. The fact that absorption of the serum by a suspension of epidermal cells prevents the reaction whereas absorption with a homogenate of tissues from other organs does not affect the character of the reaction is evidence of the organ specificity of the reaction of normal serum with epidermal antigens. This observation in turn is proof that the reaction of normal serum with antigens of differentiated epidermal cells is immunologically specific. The presence of antibodies against antigens of differentiated epidermal cells in healthy human serum also was observed by Ackermann et al. [5]. According to their observations, antibodies against antigens of cells of the stratum basale of the human epidermis appear in a higher titer in burns.

The ability of healthy human blood serum to react with antigens from different organs and tissues has been noted more than once. For instance, serum from a clinically healthy subject reacts in a definite titer with components of muscle, nerve, and connective tissues [2-4], with antigens of erythrocytes [12] and lymphocytes [14], and also with certain hormones [6, 11]. The discovery of receptors for the Fc-region of the IgG molecule on cells of many tis-

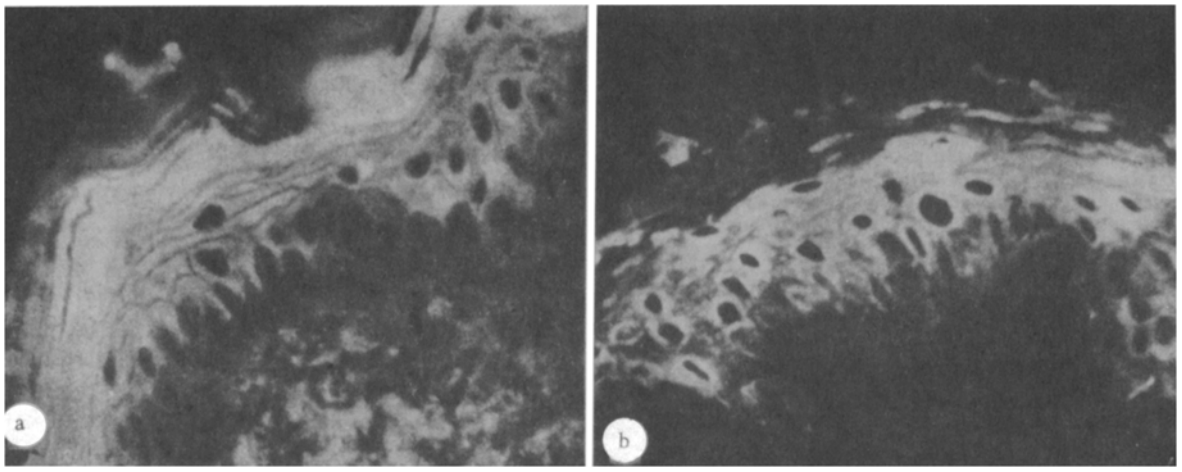

Fig. 1. Reaction of healthy human blood serum with epithelium of skin; a) intense fluorescence of cytoplasm of differentiated cells of human epidermis. No reaction with antigens of cells in stratum basale; b) reaction of healthy human blood serum with antigens of the perinuclear zone and of cytoplasm proper in cells of differentiated layers of epidermis.

sues and organs suggested that the reaction of normal sera with the components of these tissues is not immunologically specific and is due to fixation of IgG by means of the Fc-region [3]. However, it is difficult on the basis of this hypothesis to explain the specific character of absorption of normal serum, noted above, as a result of which its reaction with the epithelium of the skin is abolished only by absorption with epidermis and not by absorption of the serum with tissue homogenate from other organs.

Investigations have shown that certain antibodies, including those against antigens of epidermal cells, erythrocytes, and lymphocytes, have a complement-dependent cytotoxic action on target cells [12]. In this connection it has been suggested that they perform a catabolic function, breaking down aging cells and eliminating breakdown products from the blood stream [9, 12]. At the same time, antibodies have been shown to stimulate the growth and multiplication of cells of injured organs [1]. It has recently been shown that antibodies and their complexes with antigens play an important role in the regulation of the immune response. This suggested that antibodies present in normal serum and their complexes with tissue antigens evidently participate in the regulation of the immune response to the organism's own antigens [8, 10]. It must be noted, however, that the suggestions mentioned above provide an explanation of certain facts and, above all, the presence of a definite level of antibodies against antigens of their own tissues in the blood of clinically healthy subjects; nevertheless, most of these hypotheses still lack an adequate experimental basis, and for that reason the problem of the immunologic specificity and the physiological role of the reaction of normal serum with tissue antigens requires further study.

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IMMUNOCHEMICAL IDENTIFICATION OF FERRITIN AND ITS IMMUNOLOGIC ANALOGS β-FETOPROTEIN AND α_{2H}-GLOBULIN

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KEY WORDS: ferritin; β-fetoprotein; α_{2H}-globulin; immunochemical identification.

During the last 15 years several proteins possessing a common antigenic determinant with the ferritin of the liver have been described: a tissue α_2-globulin, β-fetoprotein, and α_{2H}-globulin [3, 6, 9]. The tissue α_2-globulin, isolated by me from human kidney tumor tissue, was indistinguishable from ferritin, and in later publications I called it ferritin [4]. The β-fetoprotein, isolated by Alpert et al., from human embryonic liver, had the electrophoretic mobility of blood serum β-globulins (ferritin has the electrophoretic mobility of α_2-globulin) and, despite its immunologic identity with the ferritin of the liver, it was classed by these workers among the human fetoproteins [6]. The α_{2H}-globulin isolated by Buff et al. from tumor tissues was very similar in its properties to ferritin but, in addition to its iron-containing fractions, it had an additional glycoprotein component which is not present in ferritin preparations [10]. This difference, these workers consider, did not allow this protein to be called ferritin [10]. As a result, some ambiguity has arisen in the terminology of ferritin in the literature, and some workers have expressed the wish either to describe the "newly discovered ferroproteins" immunologically identical with ferritin as ferritin or to argue strongly in support of giving this protein a name of its own [12].

The immunodepressive properties of ferritin [8] and the carcinogenic effect of iron on human and animal cells [11, 14] are the reasons for the undiminishing interest in ferritin, the iron depot protein in the body [7, 12, 13]. Hence the need for clear differentiation not only between the various ferroprotins, but also between the different types of ferritins, for example the isoferritins isolated from normal and tumor tissues. The object of the present investigation was to identify ferritin and its immunologic analogs described in the literature under different names.

EXPERIMENTAL METHOD

Ferritin was crystallized from normal, tumor, and embryonic tissues by Granick's method. α_{2H}-globulin was isolated by the method of Buff et al. [10]. The purity of the preparations was verified by disc electrophoresis and disc immunoelectrophoresis [2]. A preparative version of disc electrophoresis in polyacrylamide gel, the methods of immunoelectrophoresis and gel filtration in [1], and the immunodiffusion method and methods of specific detection of glycoproteins and ferroproteins in [4] also were used.

Antiferrin sera were obtained by immunizing rabbits: 1 mg ferritin with Freund's adjuvant was injected subcutaneously once a week for 4 weeks. Blood was taken on the 7th day. The antiferritin sera did not reveal antibodies against other serum and tissue proteins and they contained about 0.5 mg/ml of antiferritin antibodies. Antisera against the glycoprotein component of α_{2H}-globulin and against α_{2H}-globulin itself were obtained by the same scheme, but they contained antibodies both against the glycoprotein components and against ferritin.

The monospecific antiserum against β-fetoprotein was obtained from Alpert (USA) in 1975 and that against α_{2H}-globulin from Burtin (France) in 1973. Ferritin and β-fetoprotein were

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