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NON-COLLAGENOUS PROTEINS OF HUMAN DERMIS*

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Non-collagenous proteins of the connective tissue are represented by serum proteins and a group of proteins, not yet well characterized which are probably synthesized *in situ* and form complexes with acid and neutral polysaccharides. It is estimated, from isotope studies, that more than one half of the total plasma proteins are located in the interstitial compartment (1, 2). Albumin and various serum globulins have been identified in rabbit skin (3, 4). Fricke (5) also reported the presence of several serum glycoproteins in human Achilles tendon and synovial membranes. There is also evidence that protein-carbohydrate complexes, distinct from serum proteins, tissue fluids, etc. are present in the connective tissue (6). This investigation was undertaken in order to clarify the nature of non-collagenous proteins derived from human dermis. The main purpose of this study was (a) to estimate the amount of non-collagenous proteins (b) to identify the serum proteins and (c) to clarify the presence of proteins of local origin, distinct from serum proteins and soluble collagens.

MATERIALS AND METHODS

Twenty skin specimens were obtained from the medial incision of fresh autopsy material. Five skin specimens and their autologous blood serums were obtained from patients subjected to mastectomies or leg amputations. In another experiment the skin and autologous blood serum from six rabbits were also studied. The skins were carefully trimmed of the subcutaneous fat and the epidermis removed by stretching and scraping with a scalpel. Following lyophilization the specimens were run briefly through a Wiley micro-model mill and reduced to a fine powder. Specimens, 1 g dry weight, were extracted with 20 ml of 0.15M sodium chloride for 20 minutes in a high speed homogenizer (Vir-Tis) at 4°C. This operation was repeated four times, since a fifth extraction yielded no significant amount of proteins. Further extractions with 0.1N Na_2HPO_4, pH 8.6 yielded only minute amounts of protein. The NaCl extract was dialyzed against distilled water at 4°C for 72 hours and brought to dryness by lyophilization including the precipitate. The extracts from eight human specimens were subjected to several chemical determinations. Aliquots were hydrolyzed with 6N HCl at 110°C for 20 hours in vacuum sealed tubes and used to determine hydroxyproline (7) and tyrosine content (8). Total proteins were estimated by the method of Lowry (9). Aliquots were hydrolyzed with 4N HCl for five hours in boiling water for the estimation of hexoses (anthrone method). Hexosamines were estimated by hydrolyzing the specimens with 3N HCl for 15 hours at 110°C and the interferring substances removed with a Dowex 50 column as described by Boas (10). Sialic acids were determined by the thiobarbituric acid method of Warren (11).

The sodium chloride extracts and their autologous blood serums were analyzed by means of vertical slab acrylamide gel electrophoresis. The method used was an adaptation of the Ornstein (12) technic to the vertical slab apparatus of Raymond (13) developed in our laboratories (14). The spacer gel consisted of 3.5 per cent acrylamide, 1N HCl, Tris and TEMED, pH 6.7, while the running gel consisted of 7 per cent acrylamide in Tris-glycine buffer, pH 8.9. The extracts were used at a 3 per cent concentration and 10 microliters were run at 300 volts (110 milliamperes) for 1 hour. The gels were stained with amido black.

Immunodiffusion analysis was performed in 1 per cent Iono agar №2 (Oxoid) dissolved in Veronal buffer, pH 8.6, ionic strength 0.025. The following antibodies were tested: albumin, 7S gamma globulin, beta 2 A-globulin, beta 2 macroglobulin, orosomucoid, alpha 2 macroglobulin, transferrin, ceruloplasmin, haptoglobulins and beta lipoprotein. The extracts were also analyzed against total rabbit and horse antihuman serum (Mann Research Labs., New York). Specific antibodies against the sodium chloride extracts were obtained by injecting intramuscularly into rabbits, 20 mg of protein dissolved in 0.5 ml of physiologic saline and 0.5 ml of complete Freund adjuvant (Difco). The injections were repeated four times, at 10 day intervals, and blood serum obtained by intracardiac puncture. In order to precipitate the anti-

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TABLE I

*Chemical analysis of the 0.15N NaCl dermal extract**

mg/g dry weight

Total Proteins	Tyrosine	Hydroxyproline	Hexoses	Hexosamines	Sialic acids
24.0 ± 7	1.61 ± 0.2	0.65 ± 0.18	0.83 ± 0.08	0.89 ± 0.13	0 31 ± 0.05

* Results are reported as mean and standard deviation of eight samples.

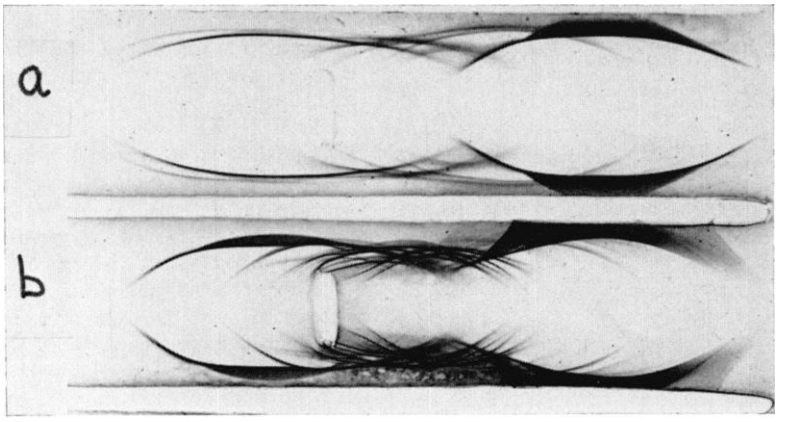

FIG. 1. Immunoelectrophoresis with horse antihuman serum. (a) human sodium chloride dermal extract and (b) autologous serum. Note the splitting of the gamma globulin in the dermal extract.

bodies against serum proteins the specific antiserum was absorbed with an excess of pooled human serum proteins. Immunoelectrophoresis was carried out in glass plates, 3¼ × 4 inches overlaid by 1 per cent Iono agar #2 in Veronal buffer, pH 8.6, ionic strength of 0.025. The samples were analyzed against total anti-human serum and absorbed and non-absorbed specific antisera.

RESULTS

The chemical analysis of the sodium chloride extract is detailed in table I. Most of the soluble proteins of human dermis are extracted with 0.15M sodium chloride and represent about two and a half per cent of the total dry weight. This extract contains about 20 per cent collagen, calculated on the basis of hydroxyproline content. There were significant amounts of tyrosine, hexoses, hexosamines and sialic acids, thus suggesting a complex of glycoprotein. Immunodiffusion analysis revealed the following serum proteins: 7S gamma globulin, beta 2 A globulin, beta 2 macroglobulin, albumin, fibrinogen, orosomucoid, alpha 2 macroglobulin, transferrin, ceruloplasmin and haptoglobulins. Beta lipoprotein was absent.

Immunoelectrophoresis of the sodium chloride extracts against total anti-human serum revealed 10 proteins which were distributed as follows: albumin, two alpha 1 globulins, three alpha 2 globulins, two beta globulins and a double line for the 7S gamma globulin (figure 1). Immunoelectrophoresis with absorbed specific antisera revealed the presence of two non-serum proteins with electrophoretic mobility of beta and gamma globulins, respectively (figure 2). Acrylamide gel electrophoresis disclosed a large number of serum proteins. The pattern of protein distribution revealed a great variability among different specimens. This was expected since serum proteins analyzed by acrylamide gel electrophoresis show a great variety of patterns among different individuals. However, there were two significant findings in the skin extracts. The skin albumin showed a faster mobility than the corresponding serum albumin and suggested a complex of two to three fractions. Also, the skin extract revealed a significant band located in front of the transferrins and behind the albumin (see figure 3). This fraction does not seem to be present in human serum.

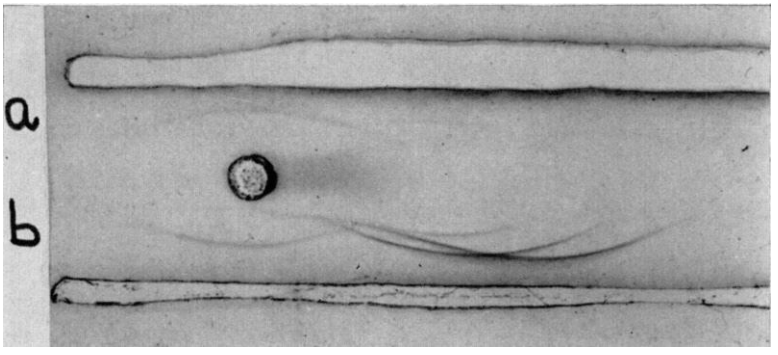

FIG. 2. Immunoelectrophoresis of sodium chloride human dermal extract. Antisera: rabbit antidermal extract. (a) absorbed (b) non-absorbed.

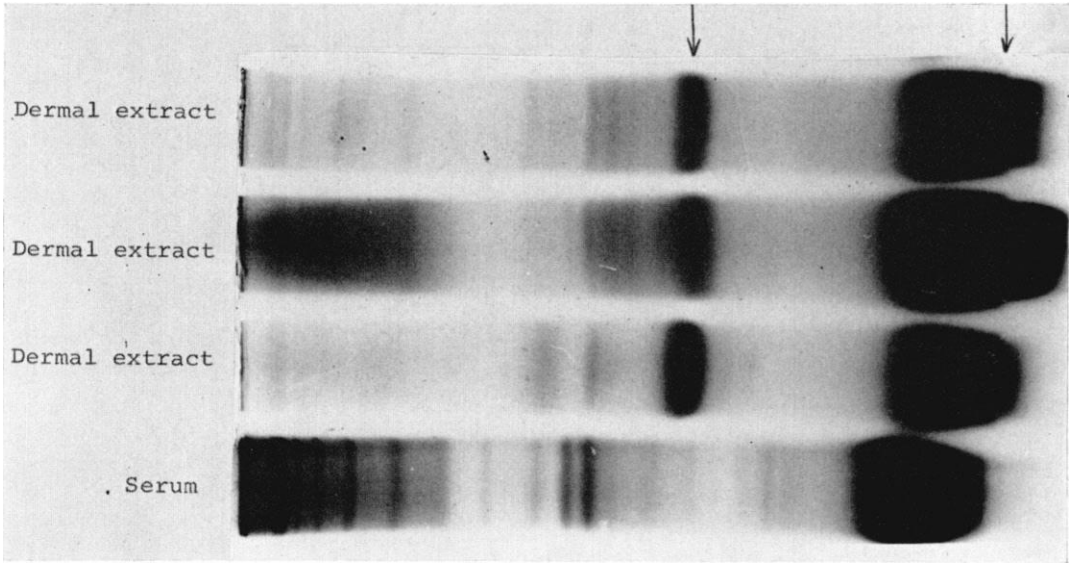

FIG. 3. Acrylamide gel electrophoresis, pH 8.9 of human specimens. Note faster mobility of the "skin albumin". There is a large fraction between the transferrins and albumin, probably of local origin.

COMMENTS

Soluble non-collagenous proteins of human dermis represent about two and a half per cent of the total dry weight. These proteins are soluble in 0.15N NaCl (physiologic saline) and are composed of serum proteins and a group of proteins which are probably products of local synthesis by connective tissue cells. Neuberger (4) has estimated, in rabbit skin, that the concentration of plasma proteins is about 1 per cent of the wet weight and that accounts for about 25 per cent of the total extravascular plasma proteins. Moreover, that study also revealed that about 90 per cent of the serum albumin in the skin was present in the interstitial compartment while only 10 per cent was located intravascularly. On the basis of this data it is quite possible that the proteins obtained in our extracts are mostly constituents of the interstitial compartment. The immunodiffusion studies showed that most of the serum proteins tested, with the exception of beta lipoprotein were present in the NaCl extract. In this regard, it is interesting to note that the three human immunoglobulins (7S gamma, beta 2 A, and beta 2 M globulins) were present in the dermis. The largest protein fraction was

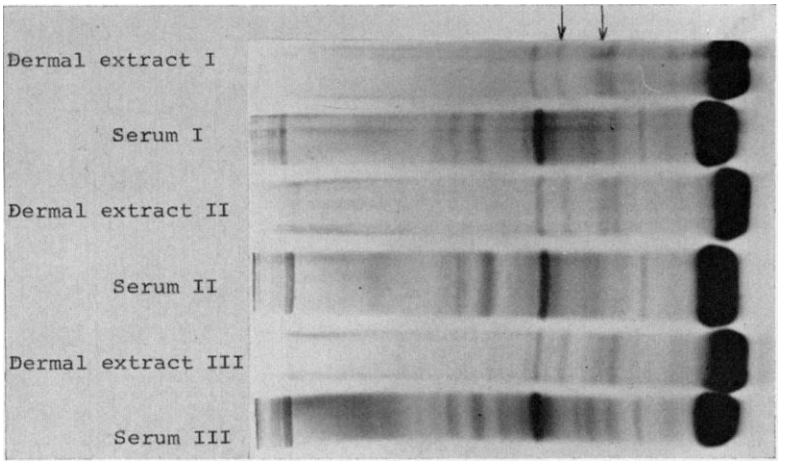

FIG. 4. Acrylamide gel electrophoresis, pH 8.9 of rabbit serum and the autologous sodium chloride dermal extracts. Note faster mobility of the "skin albumin" and two fractions (arrows) present in the dermal extract alone.

represented by the "skin albumin". This component showed consistently a faster mobility than the serum albumin when studied by acrylamide gel electrophoresis. In some extracts it was also noted that the "skin albumin" split into two to three components. At present it is not known whether the "skin albumin" represents a modified serum albumin or a complex composed of serum albumin and other proteins of local origin. Neuberger (4) isolated a "skin albumin" from rabbits and suggested that this fraction was heterogenous, consisting probably of a serum albumin and some other component synthesized *in situ*. Coleman *et al.* (15) also isolated from rabbit tendon a protein, of similar amino acid composition to that of serum albumin. However, the connective tissue protein showed a lower sedimentation constant and a faster mobility than the serum albumin in free electrophoresis at various pH. Our study also revealed a significant fraction in the NaCl extract with an electrophoretic mobility between the transferrins and the albumin. This protein was not present in numerous blood serums analyzed by us by means of acrylamide gel electrophoresis and most probably represents a local product.

Immunoelectrophoretic studies revealed the presence of two non-serum proteins with a mobility of beta and gamma globulins, respectively. This finding is similar to that reported by Fricke and Hadding (16) who studied soluble proteins extracted from human cartilage, tendon, nucleous pulposus and anulus fibrosus.

SUMMARY

It is estimated that soluble, non-collagenous proteins represent about two and a half per cent of the total dry weight of human dermis. Most serum proteins, including the three immunoglobulins have been identified. The "skin albumin" showed, by acrylamide gel electrophoresis a faster mobility than serum albumin and probably consist of a complex of several proteins. A major fraction located between the transferrins and the albumin seems to represent a protein synthesized *in situ*. Immunoelectrophoretic studies revealed the presence of two non-serum proteins with a mobility of beta and gamma globulins, respectively.

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