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Glycoproteins from Connective Tissue of Twins

EARLIER investigations on glycoproteins from connective tissue have been mainly concerned with their chemical characteristics[1-6], but very little is known about the biological function of this group of glycoproteins. Our previous observations[7] demonstrated the presence of a family of glycoproteins in cardiovascular connective tissue which varied from individual to individual and suggested a genetic basis for their differences. We have isolated glycoproteins from aorta, skin and kidney of bovine, ovine, and human twins and analysed by gel electrophoresis to determine whether variations in glycoproteins occurred among identical and nonidentical twin pairs. Because connective tissue glycoproteins also show enzymatic activities like esterase and phosphatase[8], zymograms for these two enzymes were also obtained on polyacrylamide gels. The glycoprotein samples were analysed further by immunoelectrophoresis because they are highly antigenic[3-7].

Aorta, kidney and skin tissues were collected from six bovine twin sets (two identical and four nonidentical) and from two pairs of ovine nonidentical twins immediately after the animals were sacrificed. The ages of these twin sets varied from two weeks to a year. Tissues from six pairs of human twins, two identical and four nonidentical (all immature infants, gestation period 5–6 months), were obtained from local hospitals immediately after autopsies. All the tissues were frozen at −40° C until used for extraction of glycoproteins.

Glycoproteins were isolated from the tissues by methods previously described[3], which involved extraction of the tissue with 0.15 M NaCl and precipitation of the glycoproteins from the extract at 60–100% saturation of $(NH_4)_2SO_4$. The glycoprotein sample from each tissue was analysed by electrophoresis in polyacrylamide gel in a discontinuous buffer system[9].

Electrophoresis of the samples containing 700–750 μg of glycoprotein was performed in an 'EC 470' vertical gel electrophoresis cell (E-C Apparatus, Philadelphia). A 4% 'Cyanogum-41' in Tris-HCl buffer (*p*H 6.7) was used as a spacer gel. The running gel consisted of 7% monomer in Tris-EDTA–boric acid buffer (*p*H 8.9). The same buffer system at *p*H 8.4 was used as the electrode buffer. The voltage was maintained at 200 V for 30 min and then increased to 300 V for the next 2 h. The gels were stained with amidoblack, periodic acid–Schiff (PAS) reagent[7], or with specific stains for enzymatic activity[8,10]. Glycoproteins from human twins were stained only for protein patterns (amidoblack) because of the limited availability of samples. Glycoproteins were further characterized by immunoelectrophoresis in agar[7,11]. Although antisera to each organ studied could not be prepared, rabbit antisera to glycoprotein extracts from pooled bovine aortas were used in an attempt to characterize the glycoproteins.

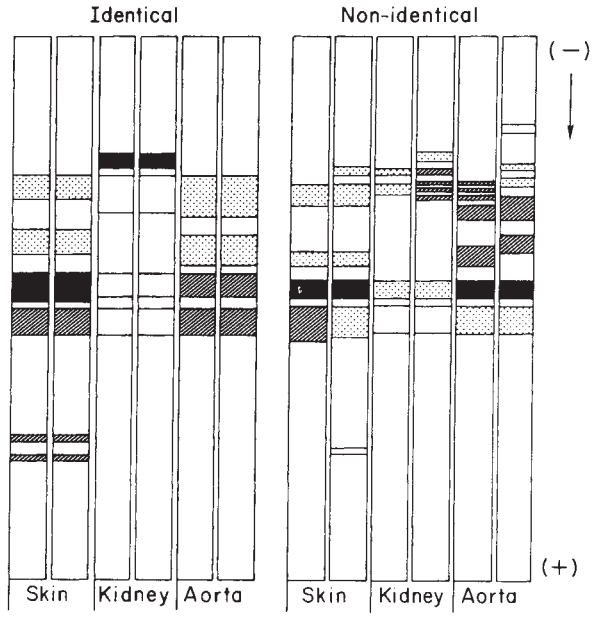


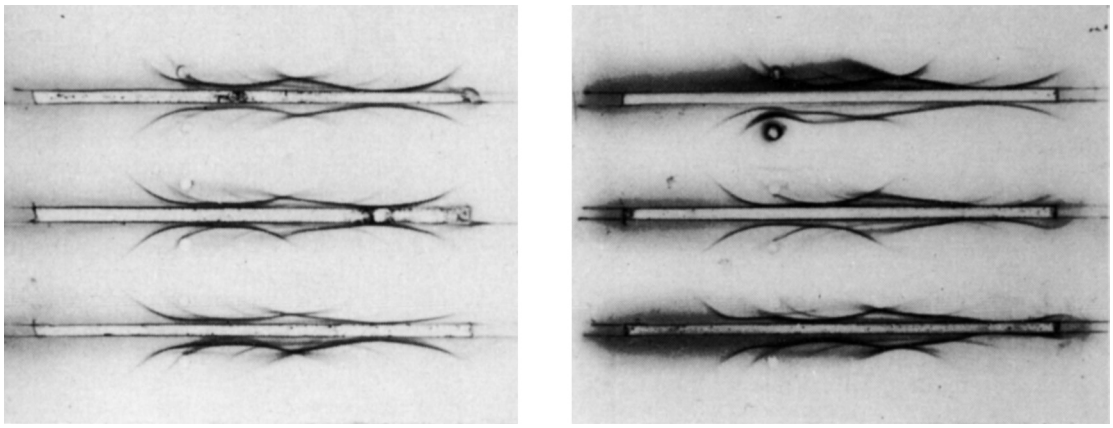
Fig. 1 Diagrammatic reproduction of polyacrylamide gel electrophoretic pattern of glycoproteins from skin, kidney and aorta of bovine twins. Electrophoresis was carried out using discontinuous gel electrophoresis technique. The gels were stained by PAS. The varying shades of the bands represent the difference in intensity produced by PAS. The dissimilarity in the pattern of glycoproteins from nonidentical twins as compared with the similarity in identical twins is apparent.

Several protein bands were observed in all samples after electrophoresis when the gels were stained with amidoblack. Similarly, the protein bands were detectable by PAS stain, indicating the presence of both protein and carbohydrate in the components being studied (Fig. 1).

All the identical twin pairs studied showed exactly similar electrophoretic patterns; but glycoproteins from different organs, skin, aorta and kidney, of the same identical twins showed considerable variations in the electrophoretic pattern for each tissue, indicating an organ specificity of these materials[7]. Furthermore, within the same species the electrophoretic pattern of one set of identical twins was found to be different from another set, which agrees with the concept of an individuality of glycoproteins[7]. Similar results were obtained with enzyme stains for esterases and acid phosphatases in the case of identical twins.

Glycoproteins from aorta, kidney and skin from the nonidentical twins, except the kidney and skin of one bovine set, showed dissimilar electrophoretic patterns when stained with amidoblack or PAS. But zymograms of nonidentical bovine and ovine twin glycoproteins showed, in most cases, quantitative rather than qualitative differences. This is in agreement with the earlier observation of Arfors *et al.*[12], who observed that only about 35% of the dizygotic human twins showed discordant zymograms for serum phosphatases. The phosphatases are glycoproteins of tissue origin and are under genetic control like other isoenzymes[13]. Quantitative differences would also be in keeping with the observation of variations of enzyme levels occurring in different clones of cells in the heterozygous state[14].

An example of immunoelectrophoretic patterns of the glycoproteins from aorta, kidney and skin of bovine identical and nonidentical twins is shown in Fig. 2. Although immunoelectrophoretic patterns of the glycoproteins from identical bovine twin pairs were similar, individual differences could be detected in the nonidentical twins. It is interesting that rabbit antibovine aorta glycoprotein gave precipitin bands against bovine kidney and skin glycoproteins, indicating the occurrence of proteins with common antigenic determinants in the organs studied. Similar cross-reactions were observed



a *b*

Fig. 2 Immunoelectrophoresis in agar of glycoproteins from aorta (upper), kidney (middle) and skin (lower) of bovine twins: *a*, identical; *b*, nonidentical. The precipitin bands were developed with rabbit antisera to glycoproteins from pooled bovine aortas (in trough).

with antisera to human sera with glycoproteins from the aorta. Because isolation and characterization of glycoproteins from connective tissue are only recent, very little is known at this time of their immunologic character.

Our earlier observation[7] that the glycoproteins in connective tissue possess macromolecular individuality is further substantiated by the present investigation. The occurrence of identical glycoproteins in connective tissues of monozygotic twins but not in dizygotic twins again suggests a genetic basis in the make-up of these compounds. Furthermore, a role for glycoproteins in transplantation rejection is suggested by the studies of Castermans[15], and more recently, murine histocompatibility antigens were shown to be glycoprotein in nature[16]. Other connective tissue compounds (collagen and mucopolysaccharides) have not shown such individual variations.

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Ultrastructural Localization of Pemphigus Autoantibodies within the Epidermis

INDIRECT immunofluorescent techniques show that the sera of patients with pemphigus vulgaris contain immunoglobulins (IgG) which specifically bind to the presumed site of the intercellular space of stratified squamous epithelia[1–3]. Pretitres of these diagnostic antibodies reflect the progress of the disease as well as the patient's response to treatment[4]. There is considerable evidence that the IgG immunoglobulins[1,5] and complement[6] found in the intercellular spaces represent autoantibodies[4]. These data imply that the distribution of the IgG deposits corresponds to the primary lesion in this disease[7,8].

The resolution of immunofluorescent microscopy, however, is not sufficient to establish whether the immunoglobulins are deposited within the intercellular space proper, on or in the cytomembranes, or in the peripheral cytoplasm of epidermal cells. Combined electron microscopic and immunocytochemical investigations were therefore undertaken We now report the first data on the actual fine structural localization of immunoglobulins within pemphigus epidermis.

Our direct immunocytochemical method makes use of an enzyme-labelled anti-human globulin antibody to reveal the site of human immunoglobulins within the tissue. Concentrated anti-human globulin antibody (obtained from Progressive Laboratories, Baltimore, Lot 2827) was conjugated with highly purified horseradish peroxidase (obtained from Schuchardt, Munich, Lot PE 029) according to the method described by Avrameas[9], using glutaraldehyde as a coupling agent. The skin of a 61 year old female patient with active pemphigus vulgaris was investigated. Samples of epidermis were removed from clinically normal and erythematous skin sites within the vicinity of bullous lesions by sliding pressure from a finger; the disease makes the skin fragile and easy to dislodge in this way (Nikolski phenomenon). The epidermal sheets were washed in phosphate buffered saline for 3 h;