FIBRONECTIN IN STRUCTURES OF TOTAL PREPARATIONS OF LOOSE CONNECTIVE TISSUE

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The use of antiserum against fibronectin has shed light on several problems connected with the function and structure of certain cells and extracellular formations of animal and human tissues [8, 11, 12]. Usually sections of tissues from organs, and cell suspensions and cultures are used as objects to study fibronectin localization [4, 5, 10]. None of this group of objects is perfectly adequate for studying the localization of tissue components during natural interaction between cellular and extracellular structures of the body, for when sections are cut, structures are divided arbitrarily, and when cells are grown *in vitro*, they often lose some of their phenotypic features or acquire new ones, untypical of them. The use of total tissue preparations has often yielded results that argue in support of their use [2].

The aim of this investigation was a comparative study of fibronectin localization in structures of the loose connective tissue of several animal species by the use of total preparations.

EXPERIMENTAL METHOD

Antiserum was obtained by immunizing rabbits with fibronectin isolated from human blood, for this glycoprotein possesses only weak species specificity [8]. Serum from rabbits immunized with a suspension of mouse fibroblasts [5] and serum of unimmunized animals were used as the control. In some experiments the sera were adsorbed beforehand with a suspension of red blood cells from animals of the species whose tissues were used for the tests (ratio 2:1). Fluorescein isothiocyanate-labeled antibodies against rabbit immunoglobulins were used in the indirect immunofluorescence test. The antibodies were isolated and labeled by methods described previously [1]. Loose connective tissue from the subcutaneous areolar tissue of mice, rats, guinea pigs, and golden hamsters was studied. The method of preparing total film preparations of loose connective tissue was described in [3]: An excised tissue fragment was stretched out by means of dissecting needles on a slide and dried in air at room temperature for 30 min. The preparations were used in the native or fixed form. After fixation in cold acetone (4°C) the slide was rinsed in physiological saline (pH 7.2), made up in phosphate buffer (BPS). The test serum was applied to the film preparation in dilutions of 1:16-1:128 and allowed to stand for 45 min at room temperature in a humid chamber. Subsequent procedures were carried out according to the method in [1]. The preparations were mounted under a coverslip in 60% neutral glycerin and examined in the LYUMAM-2 luminescence microscope. The specimens were photographed on RF-3 film with 90× objective (oil immersion) and 3× homal ocular.

EXPERIMENTAL RESULTS

After application of serum against fibronectin in dilutions of 1:64-1:128 to film preparations of mouse loose connective tissue fixed for 20 min with acetone, followed by treatment with labeled antibodies against rabbit immunoglobulins, a reaction was observed on the territory of the cytoplasm of the fibroblasts in the form of short lines and tiny dots (Fig. 1a, b) and on the surface of the thin bundles of collagen (reticular) fibers in the form of irregular deposits (glycocalyx; Fig. 1b). Sometimes the loci of the positive reaction were joined together. When unfixed preparations were tested the picture was similar but much less clearly defined. A high fibronectin concentration was observed in the region of intersection

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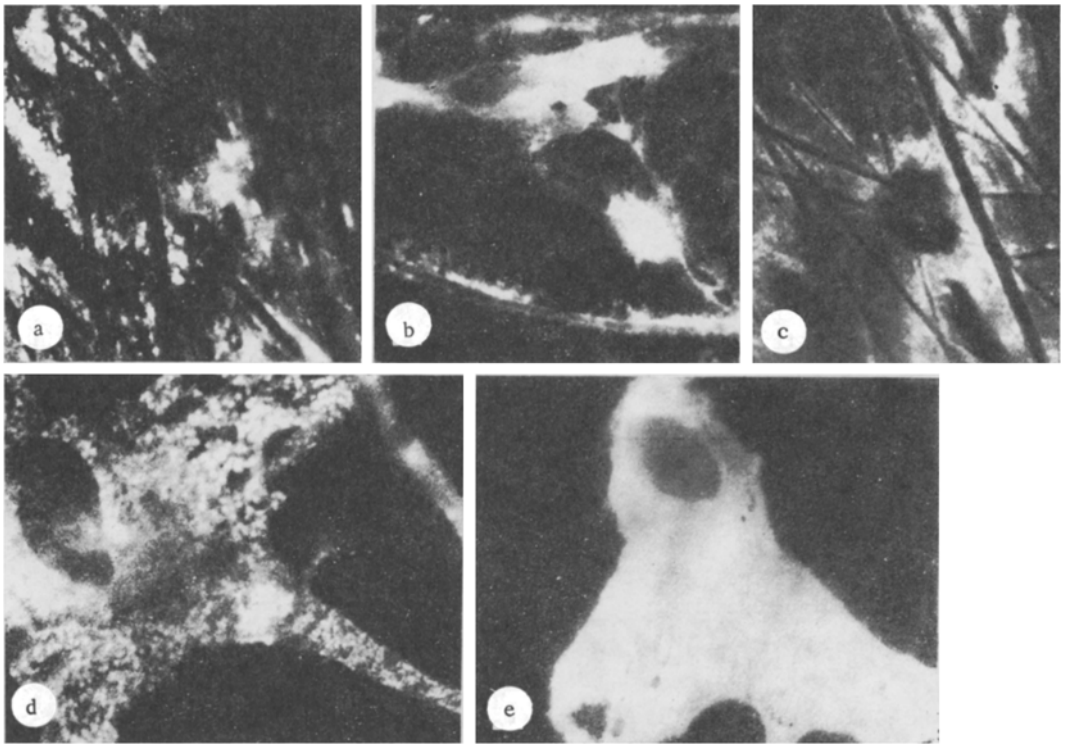

Fig. 1. Total film preparations of loose connective tissue treated with antifibronectin serum. a) Mouse tissue: reaction in cytoplasm of fibroblast consisting of pinpoint loci, serum does not react with components of elastic fibers; b) the same, reaction in cytoplasm of fibroblasts and glycocalyx of reticular fiber in the form of irregular deposits; c) the same: reaction in cytoplasm of fibroblast located at point of intersection of many fibers; d) golden hamster tissue: fibronectin localized in circular areas of cytoplasm; e) guinea pig tissue; uniform distribution of fibronectin in cytoplasm of fibroblast. Indirect immunofluorescence method, 270 ×.

of several bundles of fibers, where there were fibroblasts which apparently bound the bundles together (Fig. 1c). In loose connective tissue of hamsters, guinea pigs, and rats the localization of fibronectin was similar in character, but had a number of distinguishing features. The reaction in rats was weaker. In golden hamster fibroblasts protein was found in the form of larger loci than in mice: circular in shape and uniformly distributed throughout the cytoplasm (Fig. 1d). In guinea pigs fibronectin was diffusely distributed in the cells (Fig. 1e). Treatment of fixed total preparations of loose connective tissue with serum from unimmunized rabbits, taken in the same dilutions, gave no reaction with tissue structure components. Serum from rabbits immunized with a suspension of a culture of mouse fibroblasts reacted in mouse loose connective tissue with components of most cells but only weakly with the glycocalyx of reticular fibers. The most intensive reaction was observed with fibroblast antigens. A weak reaction was observed in the cytoplasm of macrophages. In unfixed preparations all test sera reacted intensively with cytoplasmic components of most loose connective tissue cells and did so particularly energetically with components of macrophages and mast cells. As a rule receptors for immunoglobulins are present on the surface of these cells, and they were evidently responsible for fixation of these proteins [7]. No reaction could be observed in unfixed preparations with antigens of fibrous structures.

The use of total preparations of loose connective tissue to study fibronectin localization in cells and extracellular structures thus yielded data additional to those obtained by the study of other objects. Fibronectin was found in fibroblasts of all species of animals studied (mice, rats, guinea pigs, golden hamsters), but the pattern of its distribution in the cell cytoplasm differed in different species. The protein was not distributed uniformly over the whole surface in the glycocalyx of collagen (reticular) fibers, but in the form of

deposits of different sizes. The largest concentration of fibronectin was found at points of intersection of several fibers where, as a rule, actively protein-synthesizing fibroblasts are located.

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ULTRASTRUCTURAL STEREOLOGIC ANALYSIS OF ACUTE HYPERTENSIVE MYOCARDIAL HYPERTROPHY

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A detailed explanation of the mechanisms of development of compensatory and adaptive reactions in the myocardium in arterial hypertension at the ultrastructural level is important not only for diagnosis and prognosis, but also for an understanding of general, stereotyped reactions, and also of reactions developing in the cardiomyocyte when the functional load on the heart is increased [3, 6-8]. A study of the dynamics of changes in intracellular structures and the character of their interrelations during increasing hypertension, by means of quantitative morphological methods, is of great interest in this respect.

This paper describes a study of the ultrastructure of rat cardiomyocytes hypertrophied as a result of experimental arterial (renal) hypertension, by the use of morphometric and stereologic analysis.

EXPERIMENTAL METHOD

Experiments were carried out on 35 male Wistar rats weighing initially 200.9 ± 20.3 g. To produce arterial hypertension in normotensive rats the animals were anesthetized with ether, laparotomy was performed, the region of the aorta giving rise to the renal arteries

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