INTERNATIONAL SYMPOSIUM ON

Injury, Inflammation and Immunity

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Jonathan W. Uhr, M.D.
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Preface

In the first half of this century, investigation of immunologic phenomena has been concerned with host resistance to microorganisms. In the past two decades, however, the scope of immunology has expanded dramatically with the realization that "immune" reactions have consequences far beyond their definable boundaries in infectious disease. These consequences dictate that a proper expression of the immune mechanism may be essential for the functional integrity of the body and that breakdowns in this mechanism may cause disease. It would be difficult to ascribe this shift in viewpoint to any one development of the past 20 years, but it is probably fair to say that it owes its origin, in part, to the discovery of immunologic tolerance and progress in the areas of transplantation biology, autoimmunity and other types of immunologically induced tissue damage. Tissue reactions that are due to serum antibody, moreover, can now be studied under well defined experimental conditions because of the introduction by immunochemists of precise analytical techniques. Developments in a number of areas in recent years have permitted differentiation of tissue damage produced experimentally by several types of immune reactions. Lesions of arteritis and glomerulonephritis can be induced by soluble antigen-antibody complexes; release of pharmacologically active agents, such as vasoactive amines, can be provoked by the interaction of antigen and tissue "fixed" antibody; and the study of parenchymatous tissue injury induced by delayed type hypersensitivity reactions has been stimulated by the addition of many new experimental models. These advances have resulted in a considerable accumulation of facts such that the character of current immunologic research is hardly recognizable by the classic criteria that ushered in this flourishing area of biological investigation.

One result of the mounting evidence which has led to a broadening of the concept of immunology has been the mobilization of interest in this area among investigators concerned with tissue damage and aware of but not committed to the immunologic viewpoint. Investigators have been compelled, by the complex twists and turns of research that unite one field with another, to consider the ramifications of immune reactions on tissue damage, for example, the creation and release in damaged tissue of substances that are potentially antigenic. At the same time the immunologist has grasped the opportunity to use immunologic tools to study the function of

cells in the broadest biological sense. He has shifted his emphasis from the narrow immunological viewpoint to one which permits the use of immunologic end points as measures of cellular dynamics. Thus the nonimmunologically oriented student of tissue injury and the immunologically oriented student of antigen-antibody reactions have discovered that they share wider and wider areas of common interest with an enriching of two viewpoints, once seemingly widely separated but now in close juxtaposition, if not indeed merging with each other.

In the light of this, it seemed desirable to bring together investigators whose primary interests have been focused on tissue injury and inflammatory reactions to see how their findings may bear on rather precisely defined immunologic studies. The symposia at which the papers in this book were presented provided a setting for representatives of a variety of disciplines—physiologists, pharmacologists, biochemists, microbiologists, anatomists and immunologists—to be brought under one roof to sort out the common, and uncommon, threads of their experimental experiences. The main issue concerned the step by step analysis of various types of tissue injury following both nonspecific and immunologically specific trauma. For this reason, *Injury, Inflammation and Immunity* was chosen as the title of the symposia, thus offering a relatively broad base for discussion. There was a certain overlapping of material, but in general the first symposium in London dealt with the character of physical and chemical injuries and the mechanisms underlying inflammatory changes attending such injuries; the second meeting in Mexico City concerned itself with some of the clinical aspects of immune type reactions, and the final meeting in Elkhart was devoted to immune mechanisms that lead to tissue injury.

To achieve coherence of the three symposia in the printed volume, the material has been aligned somewhat differently from the original presentations. A certain amount of new or only recently available data were offered at the symposium, but of equal significance were those presentations that recognized that much of the material in immunology is in flux and attempted to point to the direction of future research in this area. It is hoped that such an approach will offer challenges to old hypotheses and provide a framework for the synthesis of new concepts and new experimental approaches.

LEWIS THOMAS
JONATHAN W. UHR
LESTER GRANT

New York City, Spring, 1963.

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PART I

Studies of Connective Tissue

CHAPTER 1

*Homeostasis of Connective Tissues**

RUY PÉREZ-TAMAYO, M.D., AND IRMGARD MONTFORT, M.D.†

The classic concept of the connective tissue as a resistant tridimensional network, made up of inert and extracellular fibrous proteins and serving only to support epithelial structures (1), is now undergoing considerable change. Until recently the simplified picture of the organism was that of a series of functionally differentiated elements (parenchyma) arranged in organs and tissues and maintained anatomically distinct by a skeleton (stroma) formed by a single type of tissue. This picture is being modified by a large and continuously growing series of observations on the biology of connective tissues, and by the subtler but more pervasive influence of general concepts such as the dynamic state of body constituents and the nature of homeostatic regulations. No less significant has been the recognition that connective tissues may be the seat of various diseases with pleomorphic pathology (2). The concept of collagen diseases (3, 4) is more in keeping with a structure more complex and dynamic than that previously assigned to the connective tissues.

The purpose of this presentation is to review some of the data available in support of the concept of homeostasis in connective tissues. The material has been selected on the basis of its relevance to the main theme and in the hope of avoiding the dreariness of simple cataloguing. Specific instances of the quantitative behavior of collagen under different circumstances will be cited in support of the thesis that the amount of this extracellular protein is regulated in the organism following the same general principles of homeostasis which have been uncovered for many other parameters in the body. Such instances are undoubtedly a limited description

* The original data reported in this paper were obtained in part with the support of a grant from the Rockefeller Foundation.

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of collagen behavior, but to the extent to which they are clearly defined they are also correspondingly clear in their inherent limitations.

The Relationship between Parenchyma and Stroma

The quantitative relationship between parenchyma and stroma in different organs is largely unexplored. It is believed that the ratio of functioning parenchymal cells to interstitial elements changes with age (5) and sex (6) and particularly in certain disease states. Increase in stromal connective tissue is implied in the term "replacement fibrosis" (7) used to designate the result of prolonged ischemia in parenchymatous organs. Thus, the balance between parenchyma and connective tissue is thought to depend in part on the relative susceptibility of both elements to variations in oxygen supply. Furthermore hormonal influences have been suggested as significant in determining both quality and quantity of connective tissues in different organs (8, 9). There is no doubt that general factors such as blood flow and endocrine secretions, by influencing the stroma, play an important role in determining the ratio of functioning parenchymal cells to connective tissue. In addition, however, preservation of normal size and shape in different organs predicates the necessity of close local integration between parenchyma and stroma. The existence of such integration can be postulated from the following observations (table 1):

1. During the first few days after section of the phrenic nerve in rats there is hypertrophy of the corresponding hemidiaphragm without change in the relative proportion of muscle to collagen (10); furthermore the ensuing atrophy of this muscle is not accompanied by a loss of the constant parenchyma to collagen ratio until after 40 days (10).

TABLE 1

Some instances of preservation of the parenchyma/collagen ratio

Animal species	Tissue	Method	References
Rat	Diaphragm	Denervation hypertrophy and atrophy	10
Rat	Thyroid	Propylthiouracil hyperplasia	11
Rat	Uterus	Pregnancy and postpartum involution	12–17
Human	Uterus	Pregnancy and postpartum involution	15, 17
Rat	Skeletal muscle	Exercise hypertrophy	18
Rat	Skeletal muscle	Growth hormone and testosterone	19
Rat	Kidney	Hypertrophy postnephrectomy	20
Human	Heart	Hypertrophy	21
Rat	Liver	Regeneration posthepatectomy	23, 24

2. Thiouracil induced hyperplasia of the thyroid in rats, and its involution after discontinuation of the drug, occur without modification in the ratio of parenchyma to collagen (11).

3. The ratio of smooth muscle to collagen remains essentially unchanged in both rat (12–16) and human (15, 17) uteri despite the large increase in weight during gestation, and the same phenomenon is observed during postpartum involution in both species (*see below*).

4. Maintenance of the muscle to collagen ratio has been observed in hypertrophy of skeletal muscle due to exercise (18), growth hormone (19), combination of growth hormone and testosterone (19) and levator ani hypertrophy induced by testosterone (19).

5. Unilateral renal hypertrophy can be produced by removal of the homolateral kidney in rats. Using this method, Montfort and Pérez-Tamayo (20) measured the amount of total protein and collagen (as hydroxyproline) in both the normal and the hypertrophic kidney. A summary of their results appears in table 2, in which it is apparent that the ratio of parenchyma to collagen is not modified by 70 per cent increase in weight.

6. Montfort and Pérez-Tamayo (21) studied the ratio of parenchyma to collagen ratio in the normal and hypertrophic human heart. Using heart muscle of the free wall of the left ventricle they found that neither age nor sex, nor the disease responsible for hypertrophy or the degree of hypertrophy were capable of altering in a statistically significant manner the relation between parenchyma and collagen in the myocardium (tables 3, 4 and 5). Some of these results confirmed data published previously by Blumgart *et al.* in the human heart (22), and Kao and McGavack in the rat heart (5).

7. Regeneration of the liver after partial hepatectomy in the rat involves all elements, including collagen, which in the end shows the same quantitative proportions as in normal liver (23, 24).

TABLE 2

Water content, collagen protein and parenchymal protein in normal and hypertrophic rat kidneys

	Normal	10 days postnephrectomy	20 days postnephrectomy
H_2O	77.6 ± 0.8*	77.5 ± 0.5	77.5 ± 0.5
Collagen†	2.76 ± 0.2	2.35 ± 0.2	2.04 ± 0.2
Parenchymal protein‡	64.5 ± 1.8	64.3 ± 1.0	63.8 ± 1.0

* Values are in per cent of dry tissue weight.

† Calculated from hydroxyproline × 7.37.

‡ Total protein minus total collagen.

TABLE 3

*Influence of age in total protein, collagen and noncollagenous protein values of human myocardium**

Age	No. of cases	Total protein	Collagen	Noncollagenous protein
20–30	9	71.64	4.76	66.88
31–40	14	72.53	4.44	68.09
41–50	10	73.53	4.40	69.13
51–60	6	70.63	4.85	65.78
>60	6	73.83	4.01	69.82

* Figures are given in per cent of dry tissue weight.

TABLE 4

*Influence of heart weight in water, total protein, collagen and noncollagenous protein content of myocardium**

Heart weight	H_2O†	No. of cases	Total protein (N)	Collagen	Noncollagenous protein
gm	%				
<300	80.16 (11)	43	72.81	4.60	68.21
300–350	80.50 (3)	13	71.53	3.93	67.60
350–400	80.70 (33)	16	71.37	4.47	66.90
400–450	80.16 (3)	12	73.83	4.76	69.07
450–500	79.60 (1)	8	71.55	4.45	67.10
>500	78.80 (1)	13	71.83	5.11	66.72

* Total protein, collagen and noncollagenous protein values are given in per cent of dry tissue weight.

† Figures in parentheses refer to number of cases studied for determination of water content.

TABLE 5

*Influence of heart disease on total protein, collagen and noncollagenous protein values in myocardium**

	No. of cases	Total protein	Collagen	Noncollagenous protein
Normal heart				
<300 gm	43	72.81	4.60	68.21
>300 gm	11	71.40	3.97	67.43
Cardioangiosclerosis	10	72.46	5.11	67.35
Rheumatic heart disease	16	72.36	5.20	67.16
Renal diseases	15	73.54	4.03	69.51
Cor pulmonale	6	69.71	4.14	65.57
Other heart diseases	4	69.52	4.87	64.65

* Figures are given as per cent of dry tissue weight.

The data summarized above suggest that the ratio of parenchyma to collagen ratio is maintained in different organs and tissues of various animal species during processes as different as growth, regeneration and the development and involution of hypertrophy and hyperplasia. Furthermore since many of these changes in organ size are unaccompanied by similar modifications in other organs of the same animal, it can be accepted that the maintenance of the ratio of parenchyma to collagen is operated at a local level. Local integration between parenchyma and stroma may result from the independent but harmonic response of these two elements to the same or similar general stimuli, or from mutual influences between each other, or even better from a combination of both general and local factors. Regulating mechanisms for intracytoplasmic components are well known, and it is established that acceleration of synthesis or inhibition of breakdown will result in a net increase in amount. But the extracellular position of collagen, together with other metabolic and structural features of this protein molecule, presents a special problem to any hypothesis on its regulating mechanisms. Control of deposition may be exerted through the fibroblast, but the removal of collagen is a baffling problem the solution of which still awaits elucidation. But even if the mechanisms of deposition and breakdown were known, these two processes occur within rigid quantitative limits which permit the maintenance of constant ratios of parenchyma to collagen in different organs, and therefore can be subject to homeostatic regulation. This argument is further supported by reference to the reabsorption of collagen.

The Reabsorption of Collagen

There are many observations dealing with the reabsorption of collagen (25, 26), which may occur under physiologic and pathologic conditions. Relevant data for some of them are summarized below.

1. Physiologic reabsorption of collagen has been documented mainly in the reproductive organs and usually under hormonal stimulation (table 6):

TABLE 6

Some instances of physiologic reabsorption of connective tissue

Animal	Organ	Method	Observation	References
Mouse	Symphysis pubis	Pregnancy	Histologic	27,30
Guinea pig	Symphysis pubis	Pregnancy	Histologic	28,29
		Relaxin	Chemical	31–33
Rat	Uterus	Pregnancy	Chemical	12–16
Human	Uterus	Pregnancy	Histologic and chemical	15,17

(*a*) The changes in the symphysis pubis during pregnancy have been studied in both mice (27) and guinea pig (28, 29). Despite Storey's interpretation of the appearance of collagen fibers under the microscope as simple edema (30), Frieden *et al.* (31–33) have shown conclusively that during relaxation there is an absolute loss of collagen from the symphysis pubis.

(*b*) Harkness *et al.* (12–14), Montfort and Pérez-Tamayo (15), and more recently Woessner (16) have shown that during gestation the rat uterus shows a progressive increase in collagen (as hydroxyproline) reaching average values of 475 per cent above estrus figures, and that immediately after delivery there is a rapid decrease in uterine collagen (fig. 1). Collagen disappearance during uterine involution is so rapid that the half-life of this protein was calculated to be 1 day in this period. Similar observations were made almost simultaneously in the human uterus by Montfort and Pérez-Tamayo (15), and Morrione and Seifter (17). In the former study it was found that collagen increased progressively during pregnancy so that at 9 months it averaged 810 per cent above basal figures, whereas 22 days after delivery it had fallen to 138 per cent (fig. 2). It has been mentioned that the proportion between muscle and collagen was maintained almost unchanged both during gestation and after delivery. Microscopic studies

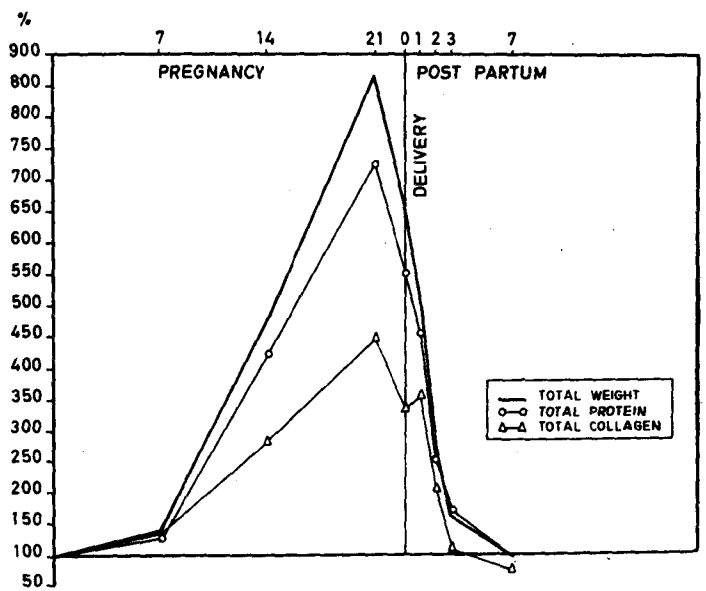

FIGURE 1. Deposition and reabsorption of collagen in the rat uterus during pregnancy and postpartum involution. Curves of wet weight and total protein are included for comparison.