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The Parenchyma/Collagen Ratio in Normal and Hypertrophic Rat Kidneys. (27634)

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Our previous work has shown that the parenchyma/collagen (P/C) ratio tends to remain constant in rat and human uteri during gestation and post-partum involution(1). In normal and hypertrophic human hearts the muscle/collagen ratio has been found to remain constant despite variations in age, sex, weight, and underlying disease(2). In view of these findings, it was considered pertinent to study the P/C ratio in other organs which would lend themselves to this type of analysis. MacKay *et al.*(3) and Addis and Lew(4) observed that after unilateral nephrectomy in the rat the remaining kidney would undergo hypertrophy for 20 days, after which weight increase continued only at the rate expected for general body growth. The highest degree of hypertrophy was 70% of normal kidney weight. The present work has explored the P/C ratio in normal and hypertrophic rat kidneys.

Material and methods. Twenty rats of Wistar strain weighing 79 to 159 g were kept in metal cages, fed a standard diet* and water *ad libitum*, and weighed daily throughout the experiment. After one week of observation they were lightly anesthetized with ether and a left nephrectomy was performed through a small lumbar incision. Groups of 10 rats were sacrificed with excess ether anesthesia at 10 and 20 days after unilateral nephrectomy, and the remaining kidney obtained. All kidneys were treated as follows: immediately after removal they were cleansed of fat, decapsulated and weighed. Total water content was determined by drying in oven at 100°C until constant weight, total protein by the Kjeldahl method, and total collagen by the technic of Neuman and Logan(5) as modified by Martin and Axelrod(6). The difference between total protein and total collagen was considered an adequate quantitative index of parenchyma, since the contribution of other proteins (elastin, smooth muscle) was probably very small.

To distinguish increase in kidney weight due to hypertrophy from that accompanying general body growth during the 20 days of the experiment, the relation of kidney weight to body weight at time of unilateral nephrectomy was expressed as a regression line(3,4, 7,8). Such regression line was extrapolated to the highest body weight reached at the end of experiment, and weights of the hypertrophic kidneys obtained at 10 and 20 days after unilateral nephrectomy were plotted in the same chart. The difference between the theoretical kidney weight for a given body weight, and the actual value encountered at time of sacrifice was the measure of hypertrophy. The regression coefficient for the correlation between kidney weight and body weight was tested for significance by dividing the regression coefficient by the standard error of the regression coefficient. This resulted

* Purina rat pellets.

in a t value which was then applied to a t table(8).

Results. The animals tolerated the procedure well and continued to gain weight throughout the experiment. This weight gain was similar in rate to the weight gain by other groups of rats of the same colony, kept in this laboratory for other purposes. Fig. 1 shows the correlation between kidney weight and body weight. Ten days after unilateral nephrectomy the average degree of renal hypertrophy was 121.6 ± 6.79%, while after 20 days it was 138.9 ± 21.3%.

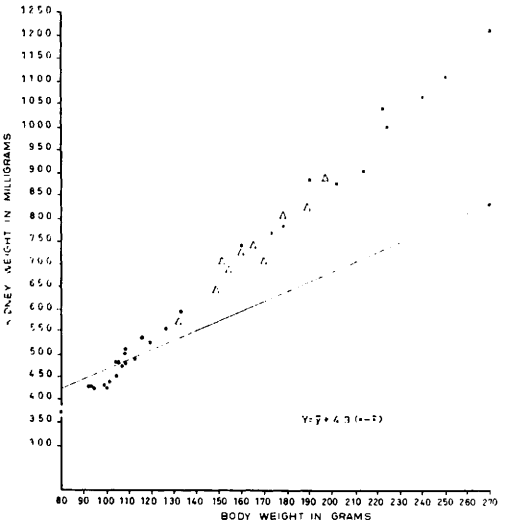

FIG. 1. Regression line of kidney weight to body weight. ●, kidney weight obtained at unilateral nephrectomy; △, kidney weight 10 days after unilateral nephrectomy; ×, kidney weight 20 days after unilateral nephrectomy.

Results of chemical determinations appear in Table I. Differences between average values of the various parameters measured in normal and hypertrophic kidneys were not statistically significant.

Discussion. It has been established(3,4, 7,8) that the relation of kidney weight to body weight is constant in rats weighing more than 40 g. The degree of hypertrophy of the kidney 10 and 20 days after unilateral nephrectomy, as calculated from the regression line extrapolated to the highest body weight achieved during the experiment, was less than that found by previous authors(3,4,7). Although a thorough study of renal hypertrophy was not the purpose of the present work, it

TABLE I. Water Content, Collagen Protein and Parenchymal Protein in Normal and Hypertrophic Rat Kidneys.*

Animal No.	H_2O	Collagen protein†	Parenchymal protein‡	H_2O	Collagen protein	Parenchymal protein
		Normal			10 days post-nephrectomy	
1	78.9	3.14	62.2	77.2	2.25	62.4
2	78.3	2.92	64.9	77.8	2.46	62.1
3	77.3	2.58	66.1	78.0	2.68	64.8
4	77.2	2.83	66.6	72.2	2.54	65.9
5	77.5	2.45	66.3	76.1	2.00	65.1
6	77.1	2.61	63.3	77.7	2.23	64.6
7	77.4	2.81	65.0	78.6	2.61	63.6
8	76.7	2.93	64.2	78.2	2.48	66.5
9	77.1	2.61	65.8	78.8	2.25	63.7
10	79.1	2.77	61.0	77.0	2.09	64.1
	77.66 ±.81	2.76 ±.21	64.5 ± 1.86	77.56 ±.57	2.35 ±.22	64.3 ± 1.40
		Normal			20 days post-nephrectomy	
11	78.3	2.52	66.8	78.5	2.04	65.1
12	78.2	2.39	66.3	77.6	2.15	64.5
13	77.7	2.71	65.7	77.6	2.08	64.4
14	78.6	2.58	62.0	77.5	1.95	63.1
15	78.2	2.23	63.7	77.1	1.92	61.5
16	78.2	2.36	65.7	77.8	1.93	63.7
17	80.0	2.67	69.1	76.5	2.14	64.7
18	77.3	2.84	65.9	77.3	2.61	63.3
19	76.9	2.11	62.6	77.8	1.77	63.2
20	78.3	2.35	64.2	77.7	1.88	64.3
	78.17 ±.83	2.47 ±.22	65.2 ± 2.11	77.58 ±.52	2.04 ±.23	63.8 ± 1.07

* All values are given as % dry tissue wt. † Calculated from hydroxyproline × 7.46.
‡ Total protein – collagen protein.

may be suggested that the lesser degree of renal hypertrophy after unilateral nephrectomy could be due to differences in the strain of animals and the composition of the diet. Nevertheless, there was an average increase in kidney weight of 21.6% 10 days after removal of one kidney, and of 38.2% after 20 days of the same operation. These figures would be correct assuming that unilateral nephrectomy does not change the normal rate of growth of the remaining kidney, and that therefore all excess in weight above normal (theorethical) values is due to hypertrophy. Such increase, on the other hand, appears adequate for quantitative determination of the P/C ratio during hypertrophy of the kidney. The data summarized in Table I indicate that there is no change in the P/C ratio, all values remaining quite close to normal at both dates studied.

It is of interest that in various other organs of different species, submitted to procedures resulting in changes in size and weight, the same preservation of the P/C ratio has been observed. Thus, in both rat(1,9,10) and human(1) uteri the smooth muscle/collagen ratio remains essentially unchanged despite the large increase in weight during gestation, and the same phenomenon is observed during post-partum involution in both species; a similar observation has been reported during development and involution of thiouracil induced thyroid hyperplasia in rats(11); during the first few days following section of the phrenic nerve in rats there is hypertrophy of the corresponding hemidiaphragm without change in the relative proportion of muscle to collagen, and this P/C ratio is maintained during involution of such hypertrophy up to 40 days, when a relative increase in collagen occurs(12); the P/C ratio remains unaltered in the quadriceps of rats undergoing hypertrophy due to excercise(13) or to growth hormone(14); hypertrophy of the levator ani produced in rats by testosterone also occurs without change in the P/C ratio(12); the P/C ratio is the same in normal and hypertrophic adult human hearts(2); regeneration of the liver after partial hepatectomy involves all elements, which upon reaching normal size show the same P/C ratio as normal livers(15, 16). It is generally accepted that both quantity and quality of connective tissues in many areas of the body are under the influence of general factors, such as oxygen supply(17) or hormonal secretions(18,19). These influences, however, are not sufficient to account for local peculiarities in the behavior of connective tissues, such as the preservation of the P/C ratio in the various organs and conditions listed above, or the several instances known where there is local reabsorption of connective tissue(19,20). Allowance should be made for the existence of local factors which participate in the regulation of the amount of stroma present in different parenchymatous organs (2). Such local factors may act independently of general stimuli, or their influence might be exerted through potentiation of the effect of variations in oxygen supply, hormones or other systemic influences.

Summary. The quantitative relation of parenchyma to collagen has been studied in normal and hypertrophic rat kidneys. The P/C ratio was defined as total protein-total collagen/total collagen. Renal hypertrophy was studied 10 and 20 days after unilateral nephrectomy. The P/C ratio was found to be the same in normal and hypertrophic kidneys. This result, together with comparable observations on the P/C ratio in different organs and various conditions, is believed to support the existence of local factors influencing the behavior of collagen in parenchymatous organs.

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Intracellular Localization of Fibrinogen.* (27635)

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Numerous reports about experimental work on cytotoxic drugs in animals, as well as clinical and pathological facts from human patients suffering liver disease, are the basis for attention to the liver and its role in the synthesis of many blood proteins. Miller and Bale, utilizing perfusion technics and radioactive isotopes, were the first to report that a physiologically normal liver produces plasma albumin and alpha and beta globulins, including fibrinogen(1). The liver is composed of multiple cell types and which cell type synthesizes any one specific plasma protein remains to be determined. We have employed immunochemical(2,3) and cellular fractionation procedures(4,5,6) to gain information on the cellular site of fibrinogen production. This protein was found intracellularly in the microsome and soluble fractions of liver parenchymal cells. The microsomal localization probably indicates the sites of synthesis while the location of fibrinogen in the soluble part of the cell represents storage.

Materials and methods. Specific antifibrinogen sera against either canine or bovine fibrinogen were produced and used to identify the cellular and intracellular localization of fibrinogen. Fibrinogen was purified from fresh plasma by ethanol fractionation followed by glycine, polyvinylpyrrolidinone and tannic acid extraction of impurities(7). Simonetti, Casillas and Pavlovsky(8) reported that tannic acid fractionation permits a complete separation of antihemophilic factor from fibrinogen. Our fibrinogen was 97-100% clottable, easily soluble after freeze drying, migrated as a homogeneous beta globulin on immunoelectrophoresis. The fibrinogen appeared to be a single antigenic component when tested against antiplasma or antifibrinogen in agar gel systems for 10 days. This fibrinogen was free of profibrinolysin, according to immunologic tests and biologic activity studies enduring 3 days after attempts were made to activate with either streptokinase or urokinase. Purified fibrinogen was mixed with $Al(OH)_3$ as adjuvant and given as a single intramuscular injection to rabbits(7). Potent antisera were produced within 10-14 days and checked by immunologic procedures to establish the individual specificity of each serum. Antifibrinogen sera were selected which did not form precipitin bands against either purified prothrombin, profibrinolysin, gamma globulin or albumin. The reactivity of the antisera was lost by adsorption with purified homologous fibrinogen. Adsorption of the antifibrinogen serum with dried whole serum did not appreciably alter the potency, and strong precipitin bands still formed against purified fibrinogen. *Agar gel diffusion systems* as modified by Wilson and Pringle (2,3) provided the immunologic evaluation. Two different kinds of cellular material were

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