

Alterations in collagen metabolism in heart and kidney on dexamethasone administration in rats

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Total collagen content in heart decreased significantly till day 8 of dexamethasone (Dex : 2.5 mg/kg week; sc for 2 weeks) treatment and increased on withdrawal of Dex. Acid soluble collagen content in heart decreased till day 12 of Dex treatment, reached normal level on day 16 of Dex treatment and exhibited an increase thereafter. Pepsin solubilized fraction in heart also behaved similarly as the acid soluble fraction, but reached normal level on Dex withdrawal. The total collagen content and the acid soluble collagen in kidney decreased significantly throughout treatment as well as on Dex withdrawal whereas, the pepsin solubilized collagen fraction in kidney exhibited a significant increase from day 8 of Dex treatment and the level was maintained throughout the experiment. Incorporation of ^{14}C -proline in both, heart and kidney was found to be reduced. Electrophoretic pattern of pepsin collagen solubilized fraction of heart and kidney revealed alterations in subunit composition and its types on Dex administration and withdrawal. Thus, administration of Dex induced alterations in the metabolism of collagen and on Dex withdrawal, the system slowly tended to attain normalcy.

Collagen is largely responsible for maintaining the functional integrity of the myocardium, which allows interdigitation and transmission of force between contracting myocytes. To correlate the collagen content with myocardial mechanical parameters, the knowledge of collagen type distribution in normal and diseased cardiac tissue is essential¹. The precise magnitude of and changes in collagen parameters may be of great significance to understand the mechanism of cardiac hypertrophy, heart failure, or pathogenesis of other cardiac diseases. Thiedemann *et al.*² show that myocardial stiffness does not correlate with total collagen content and the alteration of collagen phenotypes may be responsible for compromised function in hypertensive heart disease. Similarly in kidney, the successful formation of a relatively protein free ultrafiltrate by the glomerulus involves a complex interplay of structural, biochemical and hemodynamic factors. Kidney has its own pattern of collagen type distribution³ and any imbalance in the extracellular matrix or alterations in the metabolism of collagen in a pathological condition such as glomerulosclerosis lead to significantly reduced glomerular function⁴.

Glucocorticoids (GCs) modulate cellular functions such as energy metabolism, protein synthesis and cell proliferation which form the basis for their therapeutic

use. Glucocorticoids, synthetic and natural, markedly inhibit collagen synthesis both *in vivo* and *in vitro*^{5,6} and urinary hydroxyproline is also reported to decrease on glucocorticoid treatment⁷. Glucocorticoid treatment reduces significantly the total collagen in other cell types as well as in kidney mesangial cells but does not affect other noncollagenous protein production by mesangial cells⁴. In contrast, there are also reports demonstrating increased rates of collagen synthesis in bovine aortic smooth muscle cells^{8,9}. Dexamethasone (Dex) - a glucocorticoid is reported to interact with insulin like growth factor (IGF-1) to augment collagen production, despite having a suppressive action when added to the culture by itself¹⁰. Preferential inhibition of type III collagen is reported in one study¹¹, but another report shows a coordinated down regulation of both type I and III collagens¹². However, the effect of GCs on different collagen types has not been clearly understood. Hence, in this study, an attempt has been made to find out the effect of Dex treatment on collagen types and collagen content, its solubility as well as the incorporation of ^{14}C -proline in both heart and kidney on Dex administration and its withdrawal.

Materials and Methods

Pepsin (3630 U/mg protein), hydroxyproline and bovine serum albumin were purchased from M/s Sigma Chemical Co., St Louis, USA. All other chemicals used were of analytical grade.

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Male Wistar rats (inbred at the CLRI Animal Facility) weighing approximately 180 - 200g were housed in solid-bottomed polypropylene cages. The animals received commercial rat diet (Hindustan Lever Ltd., Bombay, India) and water *ad libitum*. The rats received, *sc*, injections of Dex at 0900 hrs at a dosage of 2.5 mg/kg/week on days 1, 3, 5, 7, 9 etc., for two weeks as described by Nasjletti *et al*¹³ with suitable modifications. Period of recovery (one week) was also included in this investigation to observe the changes following withdrawal of Dex. Age and weight matched animals which served as controls were treated with physiological saline. ¹⁴C- proline was administered, *ip*, intraperitoneally at a dosage of 40µCi/100 g body weight 12 hrs before sacrifice. For instance, the group of animals to be sacrificed on day 4 were injected with ¹⁴C-proline at 1900 hrs on day 3, i.e., on the previous day of sacrifice. Similarly, for the group of animals to be sacrificed on day 8, the injection was given at 1900 hrs on day 7, i.e., on the previous day of sacrifice and so on. The animals were sacrificed at 0900 hrs on days 4, 8, 12, 16 of treatment or on days 4 and 8 following withdrawal of Dex. Heart and kidney tissues of experimental and control animals were collected separately in ice cold saline, washed with saline and stored at -70°C until further assays.

Acid soluble collagen was extracted¹⁴. The collagen content in heart or kidney and collagen fractions were calculated by multiplying the hydroxyproline content by the factor 7.46 (ref.¹⁵). Extraction and purification of rat tail tendon collagen was carried out at 4°C as per Chandrakasan *et al*¹⁴. Pepsin soluble collagen was extracted from the heart as per Murata *et al*¹⁶.

Determination of hydroxyproline—Hydroxyproline was determined¹⁷. Samples were hydrolysed using 6 N HCl in sealed tubes at 110°C for 16 hrs. The hydrolysed samples were evaporated to dryness in a boiling water bath to remove acid. The residue was dissolved in distilled water and the solution was made up to a known volume.

Estimation of the radioactivity of ¹⁴C-proline—The radioactivity of ¹⁴C-proline was assayed as per Rojkind and Gonzalez¹⁸ with suitable modifications¹⁹. The samples were hydrolysed using 6N HCl as described previously and after neutralization with potassium hydroxide, they were made up to a known volume.

Subunit separation of pepsin solubilized collagen by SDS polyacrylamide gel electrophoresis (SDS-

PAGE)—Pepsin solubilized collagen samples were separated electrophoretically on polyacrylamide slab gel according to the method of Blackshear²⁰.

Separation of collagen phenotypes by SDS-Polyacrylamide Gel Electrophoresis (Noninterrupted)—Pepsin solubilized collagen samples were analysed by SDS- polyacrylamide gel electrophoresis (PAGE) to separate collagen types²¹.

Protein in all the samples, was assayed according to the method of Lowry *et al*²².

Statistics—The results were analysed by one way analysis of variance (ANOVA) followed by *t* test with modification of Bonferroni's method. *P* < 0.05 was considered to be statistically significant.

Results and Discussion

Corresponding control groups were carried out on all days and the mean values of all the control groups were reported, since no statistical significance in between the controls was observed.

Results are presented in Tables 1 and 2 and Figs 1 and 2.

Dex induces hypertension¹³ and affects alterations in electrocardiographic measurements²³ and in the levels of certain proteases involved in blood pressure homeostasis and blood coagulation²⁴. Hypertension, as an individual factor, is also reported to alter collagen metabolism²⁵ and the result obtained may be the consequence of both administration of Dex and its hypertensive effect. GCs are known to induce hypertension, which contributes to certain changes in the elastic and mechanical properties, thus promoting cardiovascular damage and also inducing direct endothelial damage and significant changes in vascular tissues. In another finding, the hypertensive effect of glucocorticoid hormones has been shown to subject the heart to mechanical overload which is met by the compensatory growth of the heart²⁶.

GCs have been shown to inhibit collagen synthesis in many experimental models²⁷. Corticosteroids, in general, are reported to decrease the collagen content and synthesis in various tissues. The present investigation shows that Dex treatment reduces the collagen content both in heart and kidney. The decrease in the collagen content observed in this study may be explained on the basis of hypertrophy²⁶ of both the organs viz. heart and kidney, arising due to increase in total protein content on treatment with Dex. GCs are also reported to bring down the level of the neutral salt soluble collagen content, which represents the newly synthesized/degraded collagen^{28,29}.

Table I — Collagen content and its solubility pattern, changes in α and β chains of type I collagen and changes in percentage of collagen types in heart and kidney during Dex administration and its withdrawal[Values are mean $\pm$ SE of 6 determinations (i)/4 samples (ii) and (iii)]

		Treatment Period					Recovery Period	
		Control	Day 4	Day 8	Day 12 its	Day 16	Day 4	Day 8
(i) Collagen content and its solubility pattern (μg collagen/mg protein)								
TC	H	7.38 $\pm$ 0.16	7.31 $\pm$ 0.15	6.89 $\pm$ 0.13 ^a	7.01 $\pm$ 0.14	7.14 $\pm$ 0.17	7.69 $\pm$ 0.18	8.10 $\pm$ 0.20 ^a
	K	7.19 $\pm$ 0.18	5.69 $\pm$ 0.13 ^c	5.36 $\pm$ 0.13 ^c	5.78 $\pm$ 0.14 ^c	5.19 $\pm$ 0.13 ^c	4.56 $\pm$ 0.11 ^c	4.93 $\pm$ 0.12 ^c
ASC	H	2.15 $\pm$ 0.05	1.88 $\pm$ 0.06 ^a	1.67 $\pm$ 0.05 ^c	1.76 $\pm$ 0.06 ^c	2.25 $\pm$ 0.07	2.70 $\pm$ 0.07 ^c	2.98 $\pm$ 0.07 ^c
	K	3.33 $\pm$ 0.09	2.65 $\pm$ 0.07 ^c	2.10 $\pm$ 0.05 ^c	1.80 $\pm$ 0.05 ^c	1.42 $\pm$ 0.05 ^c	1.21 $\pm$ 0.04 ^c	1.01 $\pm$ 0.03 ^c
PSC	H	5.06 $\pm$ 0.12	4.78 $\pm$ 0.10	4.47 $\pm$ 0.11 ^b	4.15 $\pm$ 0.10 ^c	3.87 $\pm$ 0.09 ^c	4.63 $\pm$ 0.11 ^a	4.98 $\pm$ 0.12
	K	2.87 $\pm$ 0.07	2.85 $\pm$ 0.07	3.16 $\pm$ 0.08 ^a	3.28 $\pm$ 0.08 ^b	3.47 $\pm$ 0.09 ^c	3.52 $\pm$ 0.09 ^c	3.54 $\pm$ 0.09 ^c
(ii) Changes in α and β chains of type I collagen (area percentage)								
β	H	29.41 $\pm$ 0.96	29.67 $\pm$ 1.01	31.82 $\pm$ 1.07	36.36 $\pm$ 1.14 ^b	28.89 $\pm$ 1.26	31.24 $\pm$ 1.43	27.00 $\pm$ 1.38
	K	30.57 $\pm$ 1.32	26.19 $\pm$ 0.99	31.40 $\pm$ 1.24	34.53 $\pm$ 1.41	35.10 $\pm$ 1.31	48.56 $\pm$ 1.95 ^c	37.91 $\pm$ 1.47 ^a
α_1	H	47.06 $\pm$ 1.88	50.01 $\pm$ 2.06	50.00 $\pm$ 2.14	47.73 $\pm$ 1.91	48.86 $\pm$ 1.96	51.24 $\pm$ 1.55	50.00 $\pm$ 1.91
	K	54.56 $\pm$ 2.13	58.63 $\pm$ 2.31	40.86 $\pm$ 1.64 ^b	47.10 $\pm$ 1.78	44.78 $\pm$ 1.88 ^a	32.50 $\pm$ 0.95 ^c	41.63 $\pm$ 1.57 ^b
α_2	H	23.53 $\pm$ 0.83	20.82 $\pm$ 0.92	18.18 $\pm$ 1.06 ^a	15.91 $\pm$ 1.15 ^b	22.20 $\pm$ 0.98	16.67 $\pm$ 1.23 ^b	25.01 $\pm$ 1.41
	K	14.86 $\pm$ 0.61	15.18 $\pm$ 0.60	27.74 $\pm$ 1.08 ^c	18.37 $\pm$ 0.76 ^a	20.05 $\pm$ 0.77 ^b	18.94 $\pm$ 0.98 ^a	20.46 $\pm$ 0.83 ^b
α/β	H	2.40	2.39	2.14	1.44	2.46	2.17	2.78
	K	2.27	2.82	2.18	1.90	1.85	1.06	1.64
(iii) Changes in percentage of collagen types (area percentage)								
Type I	H	45.36 $\pm$ 1.86	41.45 $\pm$ 1.58	39.61 $\pm$ 1.65	32.23 $\pm$ 1.26 ^b	33.25 $\pm$ 1.33 ^b	35.10 $\pm$ 1.41 ^a	46.31 $\pm$ 1.84
	K	53.33 $\pm$ 2.16	47.82 $\pm$ 1.87	41.47 $\pm$ 1.66 ^a	39.23 $\pm$ 1.53 ^b	40.00 $\pm$ 1.60 ^b	39.61 $\pm$ 1.54 ^b	36.80 $\pm$ 1.43 ^b
Type V	H	24.37 $\pm$ 1.04	22.79 $\pm$ 0.96	22.97 $\pm$ 0.92	28.44 $\pm$ 1.06 ^a	28.62 $\pm$ 1.14 ^a	25.10 $\pm$ 0.88	16.76 $\pm$ 0.59 ^b
	K	27.49 $\pm$ 1.11	29.49 $\pm$ 1.18	26.31 $\pm$ 0.99	27.10 $\pm$ 1.16	16.58 $\pm$ 0.70 ^c	12.77 $\pm$ 0.48 ^c	27.56 $\pm$ 1.10
Type III	H	30.27 $\pm$ 1.27	35.76 $\pm$ 1.43 ^a	37.42 $\pm$ 1.48 ^a	39.33 $\pm$ 1.64 ^b	38.13 $\pm$ 1.53 ^b	39.78 $\pm$ 1.61 ^b	36.93 $\pm$ 1.46 ^a
	K	19.18 $\pm$ 0.79	22.68 $\pm$ 0.89 ^a	32.22 $\pm$ 1.23 ^c	33.67 $\pm$ 1.29 ^c	43.42 $\pm$ 1.76 ^c	47.62 $\pm$ 1.85 ^c	35.64 $\pm$ 1.28 ^c
I/III	H	1.50	1.16	1.06	0.81	0.87	0.88	1.25
	K	2.78	2.11	1.29	1.22	0.92	0.83	1.03

TC = Total collagen; ASC = acid soluble collagen; PSC = pepsin solubilized collagen

H — heart; K = kidney

P values: ^a<0.05; ^b<0.01; ^c<0.001

and also to decrease the acid extractable collagen and α/β ratio in skin of neonatal rats treated with triamcinolone³⁰. A discussion is on, as to whether the hormonal effect on collagen metabolism is mainly due to a reduction in synthesis or increased degradation or a combination of both.

Earlier studies on the possible collagenolytic effect of glucocorticoids have produced contradicting results. In intact skin tissue of rats, the amount of insoluble collagen gets decreased after 14 days of treatment with cortisone²⁸. Also, later experiments with rat skin and human fibroblast cultures show increased collagenolysis after glucocorticoid treatment³⁰. In contrast, it has been reported that collagenase secretion is inhibited on treatment with GCs^{31,32}. The catabolism of newly synthesized collagen, measured as the ratio of dialysable:

nondialysable ¹⁴C-hydroxy proline, has increased considerably in the inflamed vascular wall under the effect of high and low doses of methyl prednisolone²⁸. Raghov *et al.*³³ have carried out studies to determine an important pretranslational step of Dex action on the procollagen genes and have shown that hormone treatment accelerates the rate of mRNA turnover without affecting the rate of transcription.

The decreased incorporation of ¹⁴C-proline observed in this study, estimated in whole homogenate in both heart and kidney, concurs with an earlier study in other tissues⁶. The increase in the total activity of ¹⁴C-proline observed in pepsin solubilized fraction in heart and kidney could be due to the increase in the activity of glycosylating enzymes, the galactosyl and glucosyl transferases, involved in post translational modification of collagen³⁴. Hence, the

Table 2 — Incorporation of ^{14}C -proline in heart and kidney during Dex administration and withdrawal[Values are mean $\pm$ SE of 6 determinations]

		Treatment period					Recovery period	
		Control	Day 4	Day 8	Day 12 its	Day 16	Day 4	Day 8
(i) Whole homogenate								
TA*	H	8.01±0.17	7.56±0.21	7.06±0.14 ^b	6.86±0.17 ^b	6.68±0.16 ^c	7.24±0.17 ^a	7.62±0.19
	K	13.01±0.25	12.46±0.25	11.38±0.21 ^b	11.62±0.22 ^b	11.64±0.21 ^b	11.26±0.20 ^b	12.56±0.25
SA [#]	H	1.60±0.05	1.57±0.04	1.40±0.03 ^a	1.42±0.03 ^a	1.38±0.03 ^b	1.44±0.04 ^a	1.39±0.05 ^a
	K	2.62±0.06	2.29±0.06 ^b	2.05±0.05 ^c	2.18±0.05 ^c	2.20±0.05 ^c	2.50±0.06	3.19±0.09 ^b
(ii) Acid soluble collagen								
TA*	H	3.58±0.10	3.50±0.07	2.65±0.07 ^c	2.40±0.07 ^c	2.01±0.06 ^c	3.06±0.07 ^b	3.71±0.09
	K	2.26±0.07	2.05±0.05 ^a	1.06±0.02 ^c	0.52±0.02 ^c	0.30±0.03 ^c	0.44±0.03 ^c	1.05±0.04 ^c
SA [#]	H	2.76±0.07	2.45±0.07 ^a	2.19±0.05 ^c	1.94±0.07 ^c	1.34±0.05 ^c	1.71±0.05 ^c	1.80±0.05 ^c
	K	1.15±0.02	0.87±0.02 ^b	0.63±0.02 ^c	0.38±0.04 ^c	0.28±0.05 ^c	0.48±0.04 ^c	1.40±0.03 ^c
(iii) Pepsin solubilized collagen								
TA*	H	3.40±0.08	3.93±0.10 ^b	3.98±0.10 ^b	4.08±0.10 ^b	4.06±0.11 ^b	3.78±0.09 ^a	3.62±0.07
	K	7.68±0.22	8.01±0.20	8.26±0.21	8.34±0.18 ^a	8.92±0.26 ^b	8.82±0.25 ^a	8.61±0.24 ^a
SA [#]	H	1.02±0.02	1.21±0.03 ^b	1.25±0.03 ^c	1.36±0.04 ^c	1.58±0.05 ^c	1.29±0.03 ^c	1.07±0.02
	K	3.76±0.11	3.81±0.10	3.43±0.09	3.15±0.08 ^b	3.58±0.09	3.33±0.08 ^a	3.35±0.08 ^a

TA = total activity; SA = specific activity; PSC = pepsin solubilized collagen

H = heart K = kidney

* expressed as dpm $\times 10^{-3}$ /g wet tissue.# expressed as dpm $\times 10^{-3}$ / μ mole hydroxyproline.P values : ^a<0.05; ^b<0.01; ^c<0.001

observation of increase in ^{14}C -proline incorporation in pepsin solubilized fraction in kidney may be due to increased glycosylation and due to the possibility of conversion to mature collagen. Also, the number of receptors for glucocorticoids which determines the 'tissue specific' action of glucocorticoids might also play an important role³⁵. For instance, as reported earlier³⁵, the levels of glucocorticoid receptors are different in sterna and heart and especially, the variation in the level of glucocorticoid receptors is much larger in tendon than in heart. This would suggest that the response to exogenous glucocorticoids could be different in heart and tendon during different developmental stages³⁵. Glucocorticoid receptors are the crucial factors in determining the response at tissue level³⁶. The modulation of collagen metabolism by glucocorticoids are shown to be receptor mediated in chick embryo fibroblasts and chondrocytes as described by Oikarinen *et al*⁵. Collagen production by mesangial cells is also known to be preferentially inhibited by Dex in a dose dependent manner as observed in the present study in kidney.

Although the rate of synthesis of types of collagen viz. types I, III, IV and V is reduced by Dex, the synthesis of low molecular weight collagen is

reported to be affected most dramatically⁴. Reduction of type IV collagen protein and mRNA by Dex has already been reported which describes the effect of Dex on basement membrane collagens⁴. Thus, both the interstitial and basement membrane collagens have been coordinately downregulated by Dex as described by Ohyama *et al*⁴. As reported in this investigation, the percentage of type III collagen increases throughout the experiment, while the percentage of fibrillar type I collagen gets decreased and type V does not exhibit much changes. Mukherjee *et al*³⁷ have also reported a decrease in type I: type III ratio in 10 week old spontaneously hypertensive rats. The dramatic decrease in the type I:III ratio, observed in their study, in the established phase of hypertensive hypertrophy, emphasizes that the type of collagen may play an important role in myocardial dysfunction. Thus, the decrease in type I:III ratio in kidney observed in this investigation, may also reflect the abnormalities in kidney function on treatment with Dex.

GCs are reported to decrease the synthesis of type I and type III procollagens to the same extent which indicates that Dex downregulates the synthesis of both type I and III collagens coordinately¹². Weiner *et al*³⁸ have demonstrated a direct effect of Dex on type I and type IV procollagens gene transcription as one of the

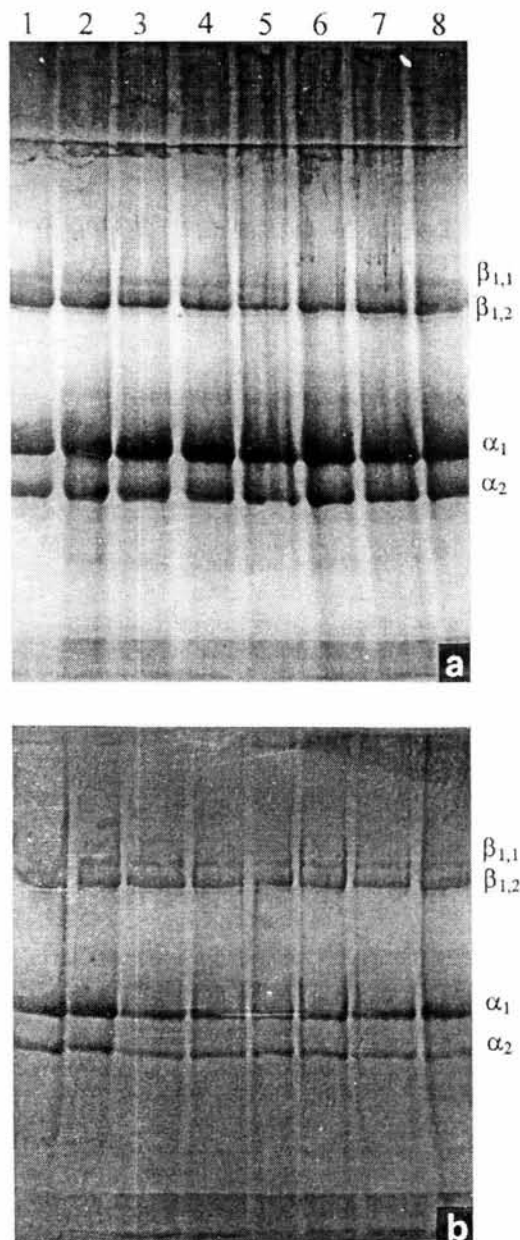


Fig. 1—Subunit separation of pepsin solubilized collagen by SDS polyacrylamide gel electrophoresis from (a) heart and (b) kidney. [Lane 1: Standard type I collagen from rat tail tendon; Lane 2: Pepsin solubilized collagen (control); Lane 3: Pepsin solubilized collagen (day 4 of treatment period); Lane 4: Pepsin solubilized collagen (day 8 of treatment period); Lane 5: Pepsin solubilized collagen (day 12 of treatment period); Lane 6: Pepsin solubilized collagen (day 16 of treatment period); Lane 7: Pepsin solubilized collagen (day 4 of recovery period); Lane 8: Pepsin solubilized collagen (day 8 of recovery period)]

several causes of steroid induced inhibition of collagen synthesis *in vivo* and *in vitro*³. Unchanged ratio of type I to type III collagens in the skin of glucocorticoid treated rats³⁰ has been reported and this suggests an equivalent effect of GCs on type I and type III collagen synthesis. The observations on the

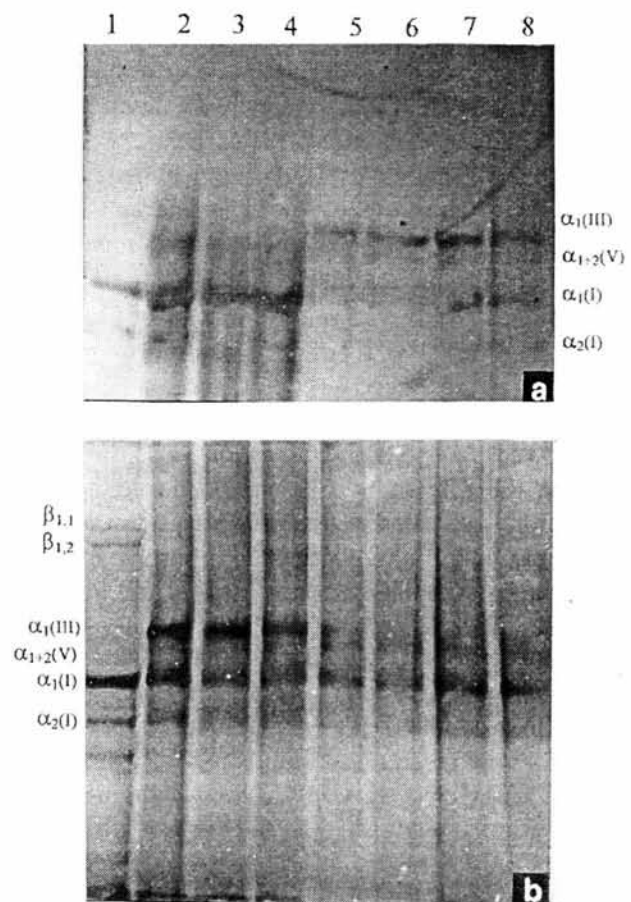


Fig. 2—Separation of collagen phenotypes by SDS polyacrylamide gel electrophoresis (noninterrupted) from (a) heart and (b) kidney. [Lane 1: Standard type I collagen from rat tail tendon; Lane 2: Pepsin solubilized collagen (control); Lane 3: Pepsin solubilized collagen (day 4 of treatment period); Lane 4: Pepsin solubilized collagen (day 8 of treatment period); Lane 5: Pepsin solubilized collagen (day 12 of treatment period); Lane 6: Pepsin solubilized collagen (day 16 of treatment period); Lane 7: Pepsin solubilized collagen (day 4 of recovery period); Lane 8: Pepsin solubilized collagen (day 8 of recovery period)]

SDS-PAGE, in this study, show an alteration in type III and type V regions which results in the decrease in the ratio of type I and type III in heart and in kidney. This may be explained on the basis that the alteration does not result from differential regulation of synthesis of procollagen types, but results from extracellular processing and/or selective extracellular degradation^{12,39}. It could be observed that heart and kidney behave differently, thus reflecting the level of receptors and the tissue specific action of GCs.

In an earlier study on the effect of Dex administration on the activity of lysosomal enzymes viz. glycohydrolases and cathepsins, the activities are found to be decreased in the initial stages of treatment with Dex in both heart and in kidney and they reach normal levels on day 16 of treatment with Dex, thus

indicating the possibility that on prolonged treatment with Dex, the system shifts from anabolic to catabolic state in both the tissues⁴⁰. The behaviour of lysosomal enzyme activity also concurs with the level of acid soluble collagen content (which represents newly synthesized /degraded collagen) which increases in heart during later stages of Dex administration, suggesting the possibility of increased degradation of collagen on chronic treatment with Dex.

The changes observed on withdrawal of Dex, may be explained on the basis of hormonal imbalance that arises on the recovery of the suppressed hypothalamic- pituitary- adrenal axis, which takes several weeks⁴¹. Future investigations should be based on the role of hypertension induced by Dex, which could be carried out by extending the treatment with Dex and by administering the antihypertensive drugs in this model to evaluate if these drugs have any effect on collagen metabolism.

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