TABLE II. Increment of HCl Output over Basal after Reserpine and Syrosingopine.

	Dose, mg	No. tests	HCl, meq/hr (increments over basal) Hr after drug 1	2	3	4
Oral						
Reserpine	.5	9	1.1	1.0	.6	1.3
"	1.0	7	1.7	3.0	4.9	4.1
Syrosingopine	.5	6	.6	.4	.4	1.7
"	1.0	10	1.5	.7	.9	1.0
Intramusc.						
Reserpine	1.0	6	1.0	2.6	6.8	7.4
Syrosingopine	1.0	6	.5	.8	1.7	2.9

Discussion. During these investigations, no observations were made regarding the primary therapeutic purpose of Syrosingopine, namely, reduction of elevated blood pressure. Consequently we do not know from first hand data whether the chemical modification of the reserpine molecule, which yields Syrosingopine, preserves the hypotensive action of reserpine. In our experience, the gastric secretagogue effect of reserpine has not been a significant problem, although it has been so reported by others(1).

Conclusion. Syrosingopine is a less potent stimulant of gastric secretion than is reserpine.

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Effect of Cortisone on Connective Tissues of the Rat.* (24329)

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Cortisone causes a decrease in the content of the hexosamine-containing mucopolysaccharides of the skin and femurs of the rat (1). However, the collagen content of these organs remains relatively undisturbed. The latter findings are related to the general metabolic inertia of the mature fibrillar components of connective tissue(2,3).

The decrease in ground substance would, of course, lead to an increase in fibrillar density. In this laboratory, the ratio of hexosamine to collagen (H/C) in tissues rich in connective tissue is used to evaluate fibrillar density. Heretofore, it has been assumed that the changes observed with skin and femurs occurred in connective tissues generally following cortisone administration. Since these cortisone-induced changes in connective tissue have certain pathological and gerontological implications, studies were made of several other tissues following cortisone administration. In addition, the changes which took place after withdrawal of cortisone were investigated.

Methods. Male rats of the Wistar or Addis-Slonaker strain were maintained on Rockland Rat Diet. Cortisone acetate (Cortone) was administered subcutaneously at a dose level of 30 mg/kg of body weight. The dose was calculated on the basis of weights obtained on alternate days. Cortisone was injected until a 20% loss occurred in body weight, at which time the animals were either sacrificed or injections were discontinued and recovery permitted to take place. When the original weight was regained, some were then sacrificed and others maintained for biopsy study.

When the response of different tissues was studied, specimens were always taken from a consistent site and in a consistent manner: 1.5 cm^2 of skin was removed from the right hind quarter of the animal and scraped free of visible fat. Approximately 100 mg of lung tissue was taken from the right lower lobe and washed free of blood by intermittent compression in saline. The sternum was cleaned free of all adhering tissue, treated with ace-

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TABLE I. H/C Ratio in Various Tissues of Wistar Rats following Cortisone Administration and Withdrawal.

Group	No.	Avg wt, g	H/C values × 10^3					
			Skin	Sternum	Trachea	Lung	Aorta	Vena cava
A	12	170	10.8 ± .3†	5.2 ± .1(8)	11.4 (4)‡	5.0 ± .1	5.5 ± .2	4.2 ± .1(8)
B	16	135*	5.9 ± .9	4.1 ± .1	6.7 (4)	3.4 ± .1	4.2 ± .1	3.5 ± .1
C	20	169	7.5 ± .2	5.3 ± .1	8.4 (4)	4.5 ± .1	5.2 ± .1	4.3 ± .1

Group A—Normal untreated rats. Group B—Rats after 35-39 days of treatment with cortisone acetate to induce 20% wt loss. Group C—Rats treated as above, then cortisone inj. discontinued and original wt regained 24-27 days later.

* Mean starting wt, 170 g. † Stand. error. ‡ When group contains fewer rats than indicated in column 2, the No. is given in parentheses.

tone-ether and dried to constant weight. Approximately 30 mg was taken for analysis. In the case of the aorta and vena cava, it was possible to obtain only 10 and 6 mg, respectively, of material.

When multiple biopsies of skin were required, specimens approximately 6 mm in diameter were taken at random from one of 6 predetermined regions of the back. The site was recorded so that subsequent biopsies were taken from another sector.

Collagen was determined by hydroxyproline analysis(4) and hexosamine by a modified Elson-Morgan procedure(5). For analysis of aorta, vena cava and the skin biopsies the modification employed for small human dermal punches was utilized(6).

Results. Following injection of cortisone, the desired weight loss took place after 35-40 days of treatment of the Wistar rats. A significant decrease in H/C ratio was induced in all of the tissues investigated. Skin exhibited the greatest decrease while vena cava showed the least (Table I). The decrease in the case of the skin appeared to be excessive. The more usual values previously observed following administration of cortisone, sufficient to cause a 20% weight loss, are 30-35%. The reason for the large fall in this case is not clear. It has been determined that a 22% loss in total body nitrogen occurs as a consequence of such treatment(7). Following discontinuation of cortisone treatment, the lost nitrogen is replaced when the animal regains its initial weight(7). After a recovery period of approximately 25 days, the original H/C values were attained for sternum and vena cava and nearly complete recovery took place in lung and aorta. The skin lagged behind considerably in this regard.

This effect was studied more fully. Multiple biopsies were taken from the backs of individual animals at the following periods of the experiment: (I) Prior to injection of cortisone acetate, (II) Following loss of 20% of body weight induced by cortisone injections, (III) Following cessation of cortisone administration, when the original weight had been regained, (IV) When an increase in body weight occurred to 20% above starting weight, and (V) When an increase in body weight occurred to 60% above starting weight. The results are shown in Table II.

For clarity of presentation, the H/C values of each set of biopsies from a single animal were compared. Thus the H/C ratio from period I for each animal was assigned an arbitrary value of 1, and the ratio of values from subsequent biopsies to that obtained at period I was derived. The mean H/C ratio decreased to 70% of the original value following a 20% weight loss induced by cortisone. A noteworthy observation was that it took almost twice as long for the Wistar rats to lose this weight as it did for those of the Addis-Slonaker strain. At the end of period III when the pretreatment weight was regained, the H/C value had recovered to only 85% of the original values. At the end of period IV, during which time the animals gained 20% in weight above the starting weight, the relative H/C values were unchanged as compared to period III. This is not according to expectation for a decrease in H/C should normally follow growth(8). Since total nitrogen content is regained at the end of period III, it must be assumed that growth had resumed. In group B relative H/C values were similar to those of animals of the same weight, but greater than their age

TABLE II. Relative H/C Values from Serial Skin Biopsies.

Group	No.	Period I Wt, g	Period I H/C	Period II T-II	Period II Wt, g	Period II H/C	Period III T-III	Period III Wt, g	Period III H/C	Period IV T-IV	Period IV Wt, g	Period IV H/C	Period V T-V	Period V Wt, g	Period V H/C
A	23	150	1	20.1	120	.71 ± .03	16.4	150	.84 ± .04	10.0	180	.83 ± .03			
	9		1			.60 ± .03			.76 ± .06			.77 ± .05			
	14		1			.79 ± .03			.89 ± .04			.87 ± .03			
Be	14	231	1	37.4	185	.70 ± .03	17.8	231	.85 ± .04	20.6	277	.87 ± .03	24.2	360	.70 ± .01
	6		1			.63 ± .02			.76 ± .06			.86 ± .03			.69 ± .02
	8		1			.77 ± .03			.92 ± .03			.87 ± .03			.71 ± .02
Ba	14	228	1				55.3	299	.86 ± .02	20.0	333	.80 ± .01	24.2	384	.72 ± .03
Bw	14										277	.88 ± .02		358	.79 ± .02

Period I: Pretreatment; Period II: following 20% wt loss induced by daily cortisone acetate inj.; Period III: following reestablishment of pretreatment wt after discontinuation of cortisone inj.; Period IV: following 20% wt gain above pretreatment level; Period V: following 60% wt gain above pretreated level.

T-II, III, IV, V: interval in days for each period prior to taking of biopsy.

A = Addis-Slonaker rats; B = Wistar rats; Be = cortisone-treated; Ba = uninj. animals of similar age; Bw = uninj. animals of similar wt during periods IV and V.

controls. However, when a 60% increase in weight was achieved by the treated animals the relative H/C values were similar to their age controls and below their weight controls.

The data were reexamined and recalculated separating groups A and B into 2 subgroups: those animals which at the end of period II exhibited a decrease in relative H/C to below 70% of the starting values and those which were above this amount. Those of Group A which showed the greatest drop in H/C also had lower values at the end of periods III and IV. In group B, a similar situation prevailed at the end of period III, but both subgroups exhibited the same values at the end of period IV. It would seem that the greater the depression in H/C, the longer is the time required for its recovery.

Discussion. Cortisone causes a decrease in H/C ratio by causing a breakdown of the hexosamine-containing ground substance(1) while disturbing little the metabolically inert mature collagen. The action of cortisone is of interest because in human beings the production of androgens diminishes markedly with age while that of the corticoids is altered less(9,10). Consequently, it would be expected that the changes in production of these metabolically opposing steroids might result in disturbances similar to those which follow cortisone administration. Indeed the H/C ratio of dermal punches from men correlates significantly with ketosteroid excretion and the product ketosteroid × ketosteroid/corticoid(11). The same disturbances in steroid production are associated with a decrease in lean-body-mass creatinine coefficient(12). Consequently, it is pertinent to ask whether the decrease in H/C which was reported previously only for skin and femur following cortisone administration is a more general phenomenon and whether or not the changes induced by cortisone are reversible.

The answer to the first question is in the affirmative. Lung, aorta and vena cava exhibit a decrease in the H/C ratio following cortisone administration although to a lesser extent than that of skin. Since loss of ground substance will influence permeability, extensibility, structure, etc., one wonders whether there is any relationship between these

changes and age-associated diseases such as hypertension, atherosclerosis, varicose veins, pulmonary emphysema and osteoporosis.

The problem of reversibility of the disturbance induced by cortisone after withdrawal of the hormone is important because it is concerned with the influence exerted by past events such as illness, surgery, and therapeutic cortisone administration upon rate of aging. Obviously information obtained on growing rats may not be applicable to human physiology but preliminary studies reveal that a decrease in H/C ratio may be observed in patients receiving therapeutic doses of cortisone for several weeks (Wright and Sobel, to be reported).

Lung, sternum, aorta and vena cava exhibit complete or nearly complete recovery of the H/C ratio following withdrawal of cortisone. Whether the original composition of the ground substance is reestablished remains to be determined.

Skin fails to reestablish the initial H/C ratio before growth continues. Apparently, the greater the depression of the H/C ratio induced by cortisone, the slower the recovery. When growth continues the changes in H/C ratio follow a complicated course. It seems however, that when the weights of the experimentally treated animals approach those of their untreated litter mates, differences in the H/C ratio no longer exist.

Summary. The effect of cortisone administration upon the hexosamine-collagen ratio (H/C) of several tissues was investigated. The H/C of skin, sternum, trachea, lung, aorta and vena cava was significantly reduced when a 20% loss in weight was induced by cortisone. When injections were discontinued and the original weight was regained, H/C of sternum and vena cava returned to starting level. This was nearly true of lung and aorta. Skin lagged behind considerably in this respect. Multiple biopsies were taken from skin following cortisone administration and withdrawal. When depression of H/C was greatest, recovery lagged the most. The findings are pertinent to the question of the general nature of cortisone effect upon connective tissue and the reversibility of this effect.

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Additional Evidence for the Existence of a New Factor Essential for Mevalonic Acid Utilization.* (24330)

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Preincubation of rat liver homogenates with crystalline ribonuclease (RNAse) was found to abolish the capacity of such homogenates to utilize mevalonic acid (MVA) for biosynthesis of nonsaponifiable material (NSF) or cholesterol (CHL)(1,2,3). Control homogenates preincubated without added RNAse ordinarily retained this biosynthetic capacity.

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