TABLE I. Effect of Evans Blue and L-Ascorbic Acid (mg/ml) in Saline (pH 7.3) on Rate of Intradermal Diffusion in Pregnant and Control Rats.

		Avg spread area (mm sq) with:								Effect of ascorbic acid (% of increase)			
		Dye alone at				Ascorbic acid at							
Avg body wt* (g)	Day of pregnancy	30	60	120	180	30	60	120	180	30	60	120	180
		min				min				min			
210	Control group	125†	145	162	174	154†	173	193	206	20	19	19	15
210	6	126	147	177	197	145	168	196	216	16	14	12	10
230	12	138	151	169	185	158	168	192	210	14	11	13	14
280	18	134	150	163	—	158	173	188	—	18	16	15	12

* Each group contains 6 ♀ rats.
† The standard deviations for various groups and times ranged between ±16 and ±24.

(control) rats by measuring the intradermal infusion rate of an Evans blue solution, alone and in combination with ascorbic acid. Dermal connective tissue permeability when tested by this method was not altered by pregnancy.

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Body and Muscle Weight Depressing Effects and Thymolytic Potencies of Glucocorticoids in the Rat. (30120)

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It is well known that the naturally occurring hormones, ACTH and cortisone, when administered at high doses for extended periods retard or arrest growth of the rabbit(1-4), monkey(5), mouse(6), rat(7-14), chick or chick embryo(15-18) and man(19,20). It has been shown that bone and bone processes are adversely affected(11,12,17,18,21-23) and that various types of degenerative changes take place in muscle tissue(1-4). When hormonal treatment is discontinued a return to normal of all modalities studied occurs(3,4,13,24,25).

In contrast, reports dealing with the effects of prolonged treatment with the newer glucocorticoids in laboratory animals are not as numerous. The effects on body weight, bone and related processes have been studied in the immature rat with triamcinolone (16α-hydroxy, 9α-fluoroprednisolone)(26,27), prednisolone (Δ^1-hydrocortisone)(26) and methylprednisolone (6α-methylprednisolone)(28). Oral administration of prednisolone or triamcinolone at doses of 1.0 and 1.1 mg/rat/day, respectively, for 3 weeks, inhibits growth as reflected by decreases in body weight and length, tibial length and epiphysial cartilage width(26,27). Subcutaneous administration of methylprednisolone produces similar effects on body weights at doses of 1.0 mg/rat/day for 2 weeks(28). In the former studies(26, 27), the authors state that the diminished food intake can account only in part for the observed changes, and that discontinuance of steroid treatment is followed by a return to normal of all parameters within 3 weeks.

That hydrocortisone, prednisolone, prednisone (Δ^1-cortisone), triamcinolone, methylprednisolone, 9α-fluorohydrocortisone, as well as dexamethasone (16α-methyl, 9α-fluoroprednisolone), and 9α-fluoromethylprednisolone also affect growth of the chick embryo is evident from the work of Karnofsky(29) and Warner and Burnet(30). These investigators have found that the relative inhibition elicited by these steroids does not agree with their respective antirheumatic activities.

Faludi *et al*(31) have shown that hydrocortisone, prednisolone, methylprednisolone, dexamethasone and triamcinolone, when administered to dogs at equipotent human antirheumatic doses, decrease body weight and size of the thighs; no degenerative changes of the muscle are observed. Cessation of steroid therapy is followed by a return to normal of the measured parameters. In addition to the aforementioned steroids, Faludi *et al*(32) have studied the effects of betamethasone (16β-methyl, 9α-fluoroprednisolone), and paramethasone (6α-fluoro, 16α-methylprednisolone) in the mouse. The usual decreases in body and muscle weights of the posterior and anterior limbs occur.

Finally, Fielder *et al*(33) have administered prednisolone and methylprednisolone each at 5.0 and 2.5 mg and triamcinolone at 5.0, 2.5 and 0.5 mg orally to dogs for several weeks; they conclude that the latter glucocorticoid is the more toxic.

In the reported comparison studies(26-33), the dose employed for any one steroid was based on relative anti-inflammatory potency reported for man. It is well known that the antirheumatic potencies of most of the therapeutically used glucocorticoids correlate poorly with biological potencies obtained in animals; moreover, the biological potency relationship which exists between any two glucocorticoids can vary from animal species to animal species(34). Hence, the question can be asked whether the increased propensity of a particular glucocorticoid to affect body and muscle weights, as reported in the literature, is merely a reflection of its greater biological activity in the animal species studied.

In an attempt to answer this question graded doses of several glucocorticoids were administered to rats for 10 consecutive days. At the end of the experimental period, potencies for each glucocorticoid, in relation to hydrocortisone with respect to effects on body weights and weights of several muscles, were calculated.

The correlation between body and muscle weight effects and the thymolytic potency of each glucocorticoid is the subject of this communication.

Materials and methods. Male Sherman rats, weighing 87-111 g, individually caged, were used. Each treatment group consisted of 8 animals. Purina rat pellets and water were supplied *ad libitum*. The glucocorticoids, hydrocortisone, prednisolone, methylprednisolone, triamcinolone and dexamethasone, suspended in a polysorbate 80-carboxymethylcellulose-saline vehicle(35) (benzyl alcohol omitted) were administered subcutaneously, on a mg/kg basis, once a day for 10 consecutive days, in a volume of 0.2 ml. Individual body weights as well as the amount of diet consumed were measured at intervals of 3 to 4 days except in pair-feeding experiments where food consumption was measured daily. At the end of the experimental period, the animals were sacrificed and the triceps brachialis, gastrocnemius and/or rectus femoris muscles removed and weighed on a Roller-Smith torsion balance.

Dose-response lines were obtained by the method of least squares. Slopes of the dose-response regression lines were combined using the reciprocals of slope variance(36). Assay precision (λ) was calculated by dividing the within assay standard deviation (s) by the slope (b) of the line(37).

Results. Paired-feeding. The hypothesis that glucocorticoids, administered at equipotent thymolytic doses, would similarly affect body and muscle weights was tested in 2 sets of experiments. Since it was known that glucocorticoids reduce food consumption, the first study was concerned with determining if this effect would be entirely or only partly responsible for the reduction in body and muscle weights. Hydrocortisone (20 mg/kg), triamcinolone (5 mg/kg), dexamethasone (0.5 mg/kg) and methylprednisolone (2.5 mg/kg) were subcutaneously administered once a day for 10 consecutive days to groups of 8 animals. These doses have approximately

TABLE I. Body and Muscle Weights* of Rats After Daily Subcutaneous Doses of Glucocorticoids for 10 Days.

Treatment	Food	Body weight (g)	Body weight Difference from control g	Body weight Difference from control % †	Triceps brachialis Wt (mg)	Triceps brachialis Difference from control g	Triceps brachialis Difference from control % †	Rectus femoris Wt (mg)	Rectus femoris Difference from control g	Rectus femoris Difference from control % †	Gastrocnemius Wt (mg)	Gastrocnemius Difference from control g	Gastrocnemius Difference from control % †	Food consumption (g/rat/day)
Control	*Ad libitum*	152 ± 11	—	—	431 ± 26	—	—	352 ± 33	—	—	600 ± 48	—	—	14.4
Hydrocortisone 20 mg/kg/day	*Ad libitum*	109 ± 12	−43	−28	274 ± 44	−157	−36	230 ± 38	−122	−35	353 ± 52	−247	−41	10.9
	Pair fed	124 ± 13	−28	−18	376 ± 55	− 55	−13	322 ± 38	− 30	− 9	536 ± 63	− 64	−10	
Triamcinolone 5 mg/kg/day	*Ad libitum*	92 ± 7	−60	−39	235 ± 22	−196	−45	197 ± 23	−155	−44	320 ± 29	−280	−46	9.9
	Pair fed	120 ± 5	−32	−21	353 ± 25	− 78	−18	313 ± 23	− 39	−11	514 ± 33	− 86	−14	
Dexamethasone .5 mg/kg/day	*Ad libitum*	106 ± 8	−46	−30	299 ± 30	−132	−31	247 ± 37	−105	−30	398 ± 62	−202	−34	10.9
	Pair fed	123 ± 8	−29	−19	365 ± 17	− 66	−15	317 ± 25	− 35	−10	521 ± 34	− 79	−13	
Methylprednisolone 2.5 mg/kg/day	*Ad libitum*	108 ± 7	−44	−29	268 ± 30	−163	−38	226 ± 25	−126	−36	353 ± 58	−247	−41	10.9
	Pair fed	123 ± 9	−29	−19	353 ± 38	− 78	−18	298 ± 27	− 54	−15	513 ± 46	− 87	−14	

* Mean ± standard deviation of 16 animals/treatment. (Pooled data from 2 experiments, each having 8 animals/treatment.)
† Differences expressed as per cent of control.

equal thymolytic effects in a 48-hour thymus test used in these laboratories(38). The amount of food consumed by each steroid-treated group at the end of each 24-hour period was calculated and made available to a corresponding group of nontreated animals. This experiment was replicated 2 weeks later. Since there were no significant differences between replicate treatments, the responses were pooled and are presented in Table I.

It was apparent that at doses which reflect equipotent thymolytic effects, all glucocorticoids markedly retarded body weight gains. The differences in body weights between control and corticoid treated animals were —43 g for hydrocortisone; —60 g for triamcinolone; —46 g for dexamethasone and —44 g for methylprednisolone. All corticoids equally affected food consumption. The average amount of food consumed during the 10-day experimental period ranged from 9.9 to 10.9 g/rat/day for the corticoid treated animals while the *ad libitum* fed control animals consumed 14.4 g/rat/day. Restricting food intake to amounts equivalent to those of the corticoid treated animals caused body weight losses which ranged from —28 to —32 g. Hence, it would appear that 53% to 65% of total body weight losses were due to diminished food intake.

All corticoids markedly depressed weights of the 3 muscles. The muscle weights of the pair-fed animals were also depressed but not so pronounced as those of the corresponding corticoid treated animals. Again, it would appear that the decreases in food consumption accounted for a considerable proportion of the muscle weight losses sustained by the corticoid treated animals. The reduction of food consumption accounted for 35 to 50% of the total reduction in weight of the triceps brachialis; 25 to 43% for the rectus femoris and 26 to 39% for the gastrocnemius.

Though the data indicated that administration of several glucocorticoids at equipotent thymolytic doses equally affected body weight, food consumption and weights of the triceps brachialis, gastrocnemius and rectus femoris muscles, it was recognized that the conclusions had been derived from a study in which a single level of each glucocorticoid had been

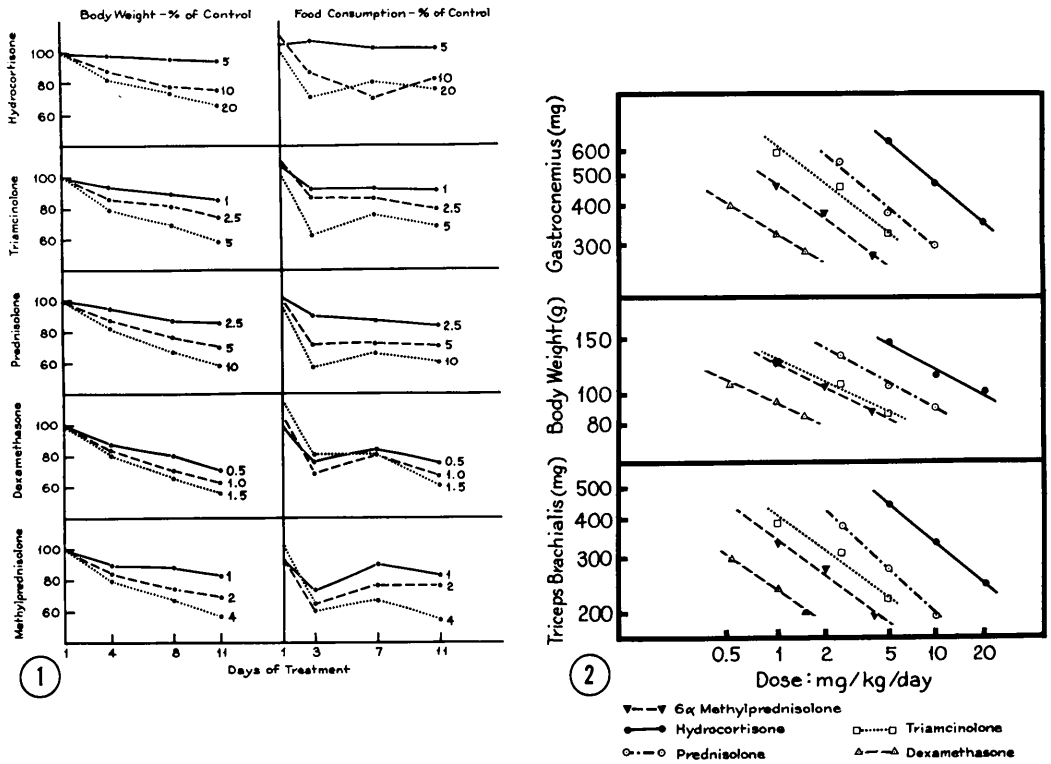

FIG. 1. Effects of daily administration of glucocorticoids (mg/kg/day, as indicated) on body weight and food consumption of the rat.

FIG. 2. Relationship between doses and body and muscle weights following daily subcutaneous administration of various glucocorticoids to the rat.

used. Hence, a second study, as shown below, was carried out in which multiple levels were studied.

Dose-response studies. Graded levels of hydrocortisone, triamcinolone, methylprednisolone, prednisolone or dexamethasone were subcutaneously administered to rats daily for 10 consecutive days. The amounts of food consumed and the effects of treatment on body weight were recorded every 3-4 days. At the end of the 10-day treatment, the animals were sacrificed, the triceps brachialis and gastrocnemius removed and weighed. Fig. 1 shows the effects of treatment on body weights and food consumption over the 10-day period. The data are presented as percentages of control. The retardation of growth was a function of both dosage and time for each of the steroids. Food consumption during the first 24 hours was irregular but then decreased rapidly during the next 2 days and remained, thereafter, fairly constant for any one dose of steroid. The average amount of food (g/rat/day) consumed during the 10-day experimental period is shown in Table II. Also given in this table and plotted in Fig. 2 are the final body and muscle weights. The dose-response relationships for body and muscle weight-decreases for each of the treatments as well as parallelism of responses among treatments are clearly apparent from Fig. 2. The potency of each steroid, relative to hydrocortisone with respect to its effects on body and muscle weights, was calculated and is presented in Table III. It can be seen that body and muscle weight depressing potencies agree quite well with the thymolytic potency of each glucocorticoid with the exception of dexamethasone. The thymolytic potency of this corticoid is about 1.7 times the body and muscle weight-depressing potencies.

Discussion. Administration of either hydrocortisone, prednisolone, methylprednisolone,

TABLE II. Final Body and Muscle Weights* of Rats Following Subcutaneous Doses of Several Steroids Once a Day for 10 Consecutive Days.

Treatment	Dose (mg/kg)	No. of animals	Body wt g	Body wt %†	Triceps brachialis mg	Triceps brachialis %†	Gastrocnemius mg	Gastrocnemius %†	Food (g/rat/day)
Control		48	150 ± 5	—	424 ± 30	—	626 ± 38	—	14.0
Hydrocortisone	5	8	144 ± 8	- 4	444 ± 27	+5	632 ± 34	+1	13.6
	10	8	115 ± 14	-23	338 ± 40	-20	462 ± 61	-26	10.7
	20	32	100 ± 11	-33	249 ± 41	-41	347 ± 47	-45	10.8
Triamcinolone	1	16	126 ± 12	-16	383 ± 43	-10	582 ± 61	- 7	12.0
	2.5	16	108 ± 9	-28	313 ± 37	-26	459 ± 58	-27	11.1
	5	32	86 ± 8	-43	221 ± 22	-48	322 ± 29	-49	9.7
Prednisolone	2.5	8	131 ± 10	-13	377 ± 32	-11	545 ± 62	-13	12.9
	5.0	8	105 ± 14	-30	271 ± 40	-36	373 ± 56	-40	10.8
	10	8	89 ± 8	-41	196 ± 33	-54	292 ± 59	-53	9.5
Dexamethasone	.5‡	16	106 ± 8	-30	299 ± 30	-31	398 ± 62	-34	10.9
	1.0	16	94 ± 5	-37	238 ± 32	-44	326 ± 34	-48	10.7
	1.5	8	85 ± 10	-43	201 ± 33	-53	285 ± 38	-54	10.8
Methylprednisolone	1	8	126 ± 7	-16	335 ± 23	-21	456 ± 20	-27	12.4
	2	8	105 ± 10	-30	276 ± 39	-35	374 ± 26	-40	10.7
	4	16	86 ± 14	-43	193 ± 34	-54	273 ± 57	-56	9.0

* Mean ± standard deviation.
† Differences expressed as per cent of control.
‡ Values taken from Table I.

triamcinolone or dexamethasone to the rat caused decreases in body weights and weights of the triceps brachialis and gastrocnemius muscles which were related to the doses of each glucocorticoid. There was no indication that body weights were more affected than muscle weights, nor was one muscle more affected than another. This was clearly seen in Table II where the differences in weights of the aforementioned parameters between control and treated animals have been expressed as percentages of control.

Each glucocorticoid, with the exception of dexamethasone, elicited decreases in food consumption which were grossly dose-related. It can only be assumed, in view of the omission of pair-fed groups, that the reduction in food consumption evinced over-all effects similar to those reported in the first experiment. It will be recalled that in the paired-feeding experiment, administration of the glucocorticoids at equipotent thymolytic doses produced comparable decreases in food consumption. This reduction accounted for a significant proportion of the total body (53 to 65%) and muscle weight losses (35 to 50% for the triceps brachialis; 26 to 39% for the gastrocnemius and 25 and 43% for the rectus femoris muscle).

Table III shows the body and muscle weight-depressing effects of each glucocorticoid, expressed in terms of potencies relative to hydrocortisone. It was evident that the relative body and muscle weight-depressing

TABLE III. Body and Muscle Weight-Depressing Effects of Steroids* in the Rat.

Treatment	Body wt	Triceps brachialis	Gastrocnemius	Thymus involution‡
Hydrocortisone	1	1	1	1
Prednisolone	3.0 (2.3- 3.9)†	3.2 (2.5- 3.9)	3.0 (2.4- 3.7)	2.2 (1.8- 2.7)
Triamcinolone	7.0 (5.7- 8.6)	5.6 (4.6- 6.9)	4.8 (4.2- 5.5)	3.8 (3.4- 4.1)
Methylprednisolone	8.3 (6.5-10.7)	8.9 (7.1-11.1)	9.1 (7.4-11.2)	7.4 (6.1- 8.9)
Dexamethasone	27.5 (21.4-35.2)	24.1 (19.6-29.4)	25.8 (20.7-32.0)	45.0 (37.0-55.0)
Average precision ($\lambda = s/b$)	.19 (.18-.20)	.17 (.15-.19)	.15 (.14-.16)	.23

* Steroids were administered once a day for 10 consecutive days, subcutaneously.
† Potencies and (95% confidence limits).
‡ Relative thymolytic potencies obtained in a single injection 48 hr bioassay in the rat(38).

potencies paralleled the relative thymolytic potency of each glucocorticoid with the exception of dexamethasone. The body and muscle weight-depressing potencies for this corticoid appeared to be significantly less than its thymolytic potency (no overlap of the 95% confidence limits in any paired comparison). More pertinent, however, was the observation that dexamethasone, whose thymolytic potency greatly exceeded that of the other corticoids, had the greatest body and muscle weight-depressing potencies. The fact that the dexamethasone potencies did not parallel each other as closely as those of the other corticoids does not invalidate the major findings of these studies: namely, the "side-effects" elicited in the rat by a particular glucocorticoid reflect its biological activity in that animal species. A similar conclusion can be drawn from the work of Steelman and Morgan(39). The authors determined the relative anti-inflammatory (granuloma inhibition), thymolytic, body weight-depressing and ulcerogenic potencies of the aforementioned glucocorticoids in the rat. Body weight-depressing potency, as well as ulcerogenic potency, correlated with the thymolytic and anti-inflammatory potencies of each glucocorticoid.

The corollary that glucocorticoids will not differ with respect to side-effects, if given at equipotent biologic doses, appears applicable to another species. Prednisolone, methylprednisolone, triamcinolone, 6α-fluoroprednisolone and cortisone were administered to children affected with perennial asthma(40,41). The doses employed were "the least amount of corticosteroid that would suffice to control asthma." With the exception of cortisone, all of the glucocorticoids suppressed linear growth to a similar extent. No definite conclusions were reached with respect to the effects of these corticoids on body weights. Using another parameter of "side-effects," Dubois *et al*(42) were unable to show differences in the incidence of peptic ulcers in patients receiving equivalent antirheumatic doses of prednisone (or prednisolone), triamcinolone, methylprednisolone, dexamethasone, cortisone or hydrocortisone.

Summary. The effects of hydrocortisone, prednisolone, methylprednisolone, dexamethasone or triamcinolone on body weights, the weights of the rectus femoris, triceps brachialis and gastrocnemius muscles and food consumption were studied in normal male rats, weighing 87-111 g. Subcutaneous administration of the aforementioned glucocorticoids, once a day for 10 consecutive days, elicited a reduction in body weights, muscle weights and food consumption which appeared roughly proportional to the relative thymolytic potency of each glucocorticoid. From pair-feeding experiments, it was apparent that the reduction in food consumption accounted for 53-65% of the total body weight losses. Muscle weight losses were similarly affected. It was concluded that glucocorticoids have similar quantitative effects on body and muscle weights of the rat if administered at equipotent thymolytic doses.

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Biological Properties of Guinea Pig Anti-Insulin Antibodies.* (30121)

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Insulin is known to be antigenic in man (1,2) as well as in animals(3,4). It was recently shown that guinea pigs may produce two types of 7S gamma globulin antibodies against the same antigen with different biological properties(5-8). It seemed of interest to determine if similar antibodies could be produced when guinea pigs were immunized with beef insulin. Moreover, an attempt was made to establish whether the oxidized B-chain of insulin had the characteristic antigenic determinants of the entire intact insulin molecule.

Materials and methods. Antigen. The insulin used in immunization of the guinea pigs consisted of zinc crystalline beef insulin (Eli Lilly Lot No. 535664).

Iletin, a mixture of zinc crystalline beef and pig insulin, was used in the serological reactions for characterization of antibodies.

Insulin B chain was kindly provided by Dr. William Konigsberg, Yale University, New Haven, Conn. in the form of pure oxidized B chain and prepared according to Craig *et al*(9).

Animals. Hartley strain albino guinea pigs

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